NIST-GCR-88-551

New Models to Assess Behavioral and Physiological Performance of Animals During Inhalation Exposures

New Name, Expanded Role
NBS became the National Institute of Standards and Technology on Aug. 23, 1988, when the Omnibus Trade and Competitiveness Act was signed. NIST retains all NBS functions and its new programs will encourage improved use of technology by U.S. industry.

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University of Pittsburgh
Pittsburgh, PA

October 1988

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Prepared for
U.S. DEPARTMENT OF COMMERCE
National Institute of Standards
and Technology
(formerly National Bureau of Standards)
Center for Fire Research
Gaithersburg, MD 20899

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75 Years Stimulating America's Progress
1913-1988

Notice

This report was prepared for the Center for Fire Research of the National Institute of Standards and Technology Under Grant Number 60-NANB4001. The statements and conclusions contained in this report are those of the authors and do not necessarily reflect the views of the National Insitute of Standards and Technology or the Center for Fire Research.

Special Report to
National Institute of Standards
and Technology
under
Grant no. 60-NANB4001
Toxicity of Plastic Combustion Products*

by

Yves Alarie, Ph.D.
Principal Investigator
University of Pittsburgh

New Models to Assess Behavioral and Physiological Performance of Animals During Inhalation Exposures

NEW MODELS TO ASSESS BEHAVIORAL AND PHYSIOLOGICAL PERFORMANCE OF ANIMALS DURING INHALATION EXPOSURES

by

Dolores Elizabeth Malek

B.S., Quinnipiac College, 1977

M.S., The George Washington University, 1983

Submitted to the Graduate Faculty of the
Graduate School of Public Health in partial fulfillment
of the requirements for the degree of
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1987

NEW MODELS TO ASSESS BEHAVIORAL AND PHYSIOLOGICAL PERFORMANCE OF ANIMALS DURING INHALATION EXPOSURES

Dolores E. Malek, Ph.D.

University of Pittsburgh, 1987

Previously the toxicity of fire smoke has been examined primarily in sedentary animals and lethality was noted. The evaluation of escape potential from a toxic environment, however, requires the measurement of sublethal responses in active animals that are escape predictive. To address this need the mouse track model and the guinea pig ergometer model have been developed.

The mouse track model was a ventilated "S" shaped exposure system. Performance was evaluated by two sublethal responses, distance traveled/time and incapacitation. At seven concentrations of CO (2500-7200 ppm) and four concentrations of HCl (1095-2095 ppm) mice were evaluated and compared to control mice. Performance was impaired within two min for both CO and HCl at all concentrations tested. A novel feature of the mouse track model was its ability to detect an early deterioration in performance before incapacitation and death.

The guinea pig ergometer model was designed where a 4.9L exposure chamber enclosed a motor driven rubberized wheel. A guinea pig ran the surface of the wheel according to a 55-minute protocol. Distance traveled before incapacitation as well as respiratory frequency, tidal volume, oxygen uptake and carbon dioxide output were measured in control animals and groups of guinea pigs exposed for 30 min to 2,200 or 8,290 ppm CO. The distance traveled was noted in individual animals exposed to HCl (411, 530, 572, 591 or 652 ppm). Six groups of sedentary animals were exposed for 30 min to CO (5,700-19,000 ppm).

From the evaluation of CO and HCl, the toxicity of a given exposure condition increased with exercise. Compared to the guinea pig, the human is twice as sensitive to CO and at least as sensitive to HCl. Extrapolation of exercising guinea pig data to human was similar to theoretical models that predict human response to CO. Humans were estimated to progress five times the distance of the guinea pig at a similar level of toxicity for CO.

By distance traveled and incapacitation, the guinea pig ergometer model can evaluate pure gases, mixtures, and products of combustion. This type of data is critical to fire smoke toxic hazard evaluation.

FORWARD

TO JUDE

I am grateful to the five members of my dissertation committee for their guidance during the course of this research effort.

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INTRODUCTION

A. Toxicity of Fire Smoke

Years of work have been dedicated toward attenuating the adverse effects of fire in vehicles of transportation, as well as commercial and residential structures. The enterprise has been complicated by the introduction of new, mostly synthetic materials into the built environment. At a recent meeting* at the National Bureau of Standards, the most recent multicomponent fire modeling techniques were described to predict the fire hazard to human occupants of burning buildings. It was obvious that data were lacking which was relevant to the performance of occupants while escaping.

Many approaches to toxicology, including the relatively new area of research on the toxicity of smoke from burning materials, have utilized lethality as a response end point (Alarie and Anderson, 1981; Levin, et al., 1982). Lethality is an effective measure for making comparisons of toxic potency among a wide range of substances without regard for mechanism and sites of action (Casarett and Doull, 1980). To better elucidate mechanisms of action and determine concentrations of fire smoke that do not necessarily produce death but rather sublethal effects, it would be useful to study performance and escape potential. If escape capability is inhibited, the victim can often be considered "as good as dead" since a rescue is required for survival. In addition,

*Annual Conference on Fire Research, Center for Fire Research, National Bureau of Standards, November, 1986.

regardless of observed lethality or sublethal effects, most fire smoke toxicity data is based on inhalation exposures to inactive animals (Alarie and Anderson, 1981; Levin, et al., 1982). Those individuals escaping from a fire are not sedentary but are working to escape, and have an increased metabolic level due to psychological, emotional and physiologic factors. Fire smoke inhalation toxicity data from active animals during escape and predictive of sublethal responses, are imperative for an effective assessment of fire toxic hazard.

B. Major Constituents in Fire Smoke

The two major toxic constituents of fire smoke are the asphyxiants, such as carbon monoxide, hydrogen cyanide, and decreased oxygen and the irritants such as hydrogen chloride, acrolein and formaldehyde. Various animal behavioral models have been developed to investigate sublethal responses to combustion atmospheres or to specific asphyxiating and irritating gases of such atmospheres (Table 1). In most of these models, animals were trained prior to exposure to perform a specific task. If, during the toxic exposure, the animal fails to perform this task or fails to perform it within a designated period of time, it is deemed "incapacitated." The state of incapacitation is intended to be predictive of escape in each of these model designs.

C. Models to Study the Effects of Fire Smoke

The leg-flexion shock avoidance response was originally used for fire smoke toxicity studies at the University of Utah (Packham, 1974) and during the development of the National Bureau of Standards smoke toxicity test (Levin, et al., 1982). In this model, rats were trained prior to exposure to avoid shock by keeping their leg flexed and raised above an electrified plate. During a toxic exposure if the rat failed

TABLE 1

Exposure concentrations (c) and times (t) (Ct products) of CO or HCN associated with incapacitation[a]

Subject/Method	CO Ct ppm-min	CO References	HCN Ct ppm min	HCN References
Humans	35,000-45,000 (light activity)	Kimmerle, 1974; Stewart, et al., 1973	750-2500	Kaplan, et al., 1984
Baboons (SwRI)	34,000 (active)	Kaplan, et al., 1984		
Monkeys (Huntingdon)	25,000 (active) 50,000 (restrained)	Purser and Woolley, 1983	1176-1900	Purser, et al., 1984
Rats				
Leg Flexion (Utah)	30,000-40,000	Hartzel, et al., 1983; 1976		
(SwRI)	30,000-40,000		1200-2700	Kaplan, et al., 1984
Activity Wheel (FAA)	37,000	Crane, et al., 1977	800	Crane, et al., 1977
(Michigan)	30,750	Boettner, et al., 1978		
(McDonnell Douglas)	22,000-36,000	Gaume, et al., 1981		
Pole-Climb Avoidance (SRI)	48,000	SRI, 1978		
Rotarod (Michigan)	31,500	Boettner, et al., 1978		
(SwRI)	36,000-44,000	SwRI (unpublished)		
Shuttlebox (Michigan)	41,000-53,000	Boettner, et al., 1978		
(SwRI)	39,900	Kaplan, et al., 1984		

[a] Table modified from Kaplan, et al., 1984

to avoid the shock, it was considered to be incapacitated. In pure gas studies, concentration (C) times time to incapacitation (t) (Ct) data have been established for the asphyxiants carbon monoxide and hydrogen cyanide (Hartzell, et al., 1985; Kaplan, et al., 1984).

The incapacitating effects of combustion atmospheres have also been investigated with the use of motor driven activity wheels. The Federal Aviation Administration (FAA), McDonnell Douglas Corporation and U.S. Testing Co., have used such a method. Both the FAA (Crane, et al., 1977) and the University of Michigan (Boettner and Hartung, 1978) have studied the effects of carbon monoxide on rats under such conditions.

Activity wheels that were not motor driven have been adopted by the Japanese to assess "collapse" in mice exposed to various combustion atmospheres as well as pure gases and gas mixtures (Sakurai, T., 1986, Kishitani and Nakamura, 1979). Tepper, et al. (1985) used a similar model to test the irritants ozone and ammonia; however, incapacitation was not examined but rather overall changes in rodent (mice and rat) running activity.

Another model that has been used to study sublethal effects of inhaled combustion atmospheres and pure gases is the pole-climb conditioned avoidance/escape test (Parent, et al., 1979; Dilley, et al., 1978) which was developed at SRI International. This uses a conditioned avoidance response (CAR) before and after exposure to monitor sensorimotor performance. A rat is required to climb a greased pole in order to avoid or escape shock delivered from a grid floor below the pole. Failure to escape shock reflects a state of incapacitation.

Investigators at the University of Michigan (Hartung, et al., 1977; Boettner and Hartung, 1978) used a rotarod performance method to assess

sensorimotor deficits in rodents exposed to combustion products and carbon monoxide. Animals in this model are required to balance on a motorized rod. During a toxic exposure, a fall from the rod and failure to remount the rod in a designated period of time reflects incapacitation.

Investigators at the Southwest Research Institute have studied the effects of carbon monoxide and hydrogen chloride on both rats and baboons, as well as the effects of acrolein on baboons (Kaplan, et al., 1985). A commercially available shuttlebox was used to evaluate escape cabability in rats exposed to toxic environments. The shuttlebox consisted of two identical chambers separated by a motor-operated partition. A rat was considered to have escaped from a toxic environment when it pressed an appropriate lever after being cued by a conditioned stimulus (tone), avoided shock and moved to a toxicant-free side of the shuttle box. The larger scale escape performance paradigm for the baboons was similar to that used to evaluate the rat; however, the primates were required to discriminate between colored lights and, select and press the appropriate lever for escape.

The mouse track model has recently been developed at the University of Pittsburgh (Malek, et al., 1987). In this model, mice were trained to run in an air ventilated 150 foot "S" shaped tubular exposure track and their individual distance traveled per unit of time was recorded. The tube system was then equilibrated with a known concentration of toxicant, and the mouse was reintroduced. Not only was the occurrence and time of incapacitation recorded but also distance traveled/time, a sublethal response uniquely assessed in this model. This system was used for the evaluation of carbon monoxide, low oxygen, and hydrogen

chloride. An overall deterioration in running performance could be observed during toxic exposures compared to air controls.

Other models that measure behavioral change, although not incapacitation, during exposure to components of fire atmospheres include the irritant escape model of Wood (1979) and escape activity model of Matijak-Schaper and Alarie (1982). Wood (1979) quantified the escape response in mice exposed to ammonia. These animals prevented their exposure by breathing available humidified air or responded by actually terminating the delivery of ammonia. Matijak-Schaper and Alarie (1982) developed a model where mice were placed in a body plethysmograph, and escape activity as well as respiratory pattern (indicative of irritation, asphyxiation or death) were analyzed. Escape activity was defined in this model as movements of the mouse within the plethysomograph that produce rapid and high amplitude changes in pressure. The effects of the asphyxiants carbon monoxide, hydrogen cyanide and low oxygen were examined in this model. Although body movements were monitored and valuable respiratory responses were evaluated, exposures were to sedentary animals.

All of the animal models described, including the more recent fire smoke escape models of Kaplan, et al., 1985 and Malek, et al., 1987, have varied in their sensitivities to evaluate asphyxiants versus irritants.

Most fire fatalities due to smoke inhalation have been attributed to carbon monoxide poisoning (Halpin, et al., 1978). Carbon monoxide exerts its toxicity by interfering with the oxygen transport function of the blood by combining with hemoglobin (Hb) to form carboxyhemoglobin (COHb). Its affinity for Hb is approximately 240 times compared to

oxygen. In addition to reducing the oxygen carrying capacity for Hb, the presence of COHb interferes with the unloading of O_2 (West, 1979). Most of the animal models described respond well to the asphyxiating effects of carbon monoxide. Animals, regardless of model used, are incapacitated at a Ct value of approximately 35,000 ppm.min (see Table 1) depending on species of animal, its weight and level of activity (Kaplan and Hartzell, 1984). Mice are incapacitated at a lower Ct range (17,000 - 25,000 ppm.min) in the mouse track escape performance model (Malek, et al., 1987). This is to be expected since CO uptake in mice is faster than in humans given the same environmental conditions (Haldane, 1895). Respiratory minute volume ($\dot{V}_E$) is higher as a function of body weight in mice, therefore, the loading of CO is further enhanced by running.

The Ct value of 35,000 ppm. min seems to correspond to the Ct range likely to compromise human performance during physical activity. A COHb of 30-40% has been associated with symptoms which include severe headache, ennui, dizziness, weakened eyesight, nausea, vomiting and prostration (see Table 2, Kimmerle, 1974). From human COHb saturation curves also presented in Kimmerle, 1974, a Ct value range of 60,000-75,000 ppm.min is associated with 30-40% COHb. This Ct range is for an individual at rest where $\dot{V}_E$ is approximately 6 L/min (DuPont and Freedman, 1983). If the same individual were engaged in moderate activity ($\dot{V}_E$ = 20 L/min) then 35,000-45,000 ppm. min CO exposure could easily produce incapacitation. With a further increase in $\dot{V}_E$ such as that associated with escaping from a fire, an incapacitating Ct may even be lower, comparable to the range determined for mice in the mouse track model (Malek, et al., 1987).

TABLE 2

% COHb	Symptoms in Humans
0-10	None
10-20	Tension in forehead, dilation of skin vessels
20-30	Headache, pulsation in sides of head
30-40	Severe headache, ennui, dizziness, weakening of eyesight, nausea, vomiting, prostration
40-50	Same as above, increase in breathing rate and pulse, asphyxiation and prostration
50-60	Same as above, coma, convulsions, Cheyne-Stokes respiration
60-70	Coma, convulsions, weak respiration and pulse, death possible
70-80	Slowing and stopping of respiration, death within hours
80-90	Death in less than an hour
90-100	Death within a few minutes

Table from Kimmerle, 1974

The asphyxiant hydrogen cyanide is a common combustion product, released from the thermal degradation of both natural materials such as silk, wool and gelatine and synthetic materials such as nitrocellulose, modified acrylic fiber, rigid and flexible polyurethane foam and acrylonitrile butadiene styrene (Levin, et al., 1982, Kimmerle, 1973). HCN is known to react with mitochondrial cytochrome oxidase c which prevents the utilization of available oxygen, and asphyxiation results (West, 1979).

The foot flexion shock/avoidance model has been used extensively to investigate the incapacitating effects of HCN (Hartzell, et al., 1985; Kaplan, et al., 1984). From the curved portion of the Ct curve, incapacitation of rats occurs with accumulated doses in the range of 1200 to 2700 ppm. min. (see Table 1). Death occurs approximately at a two fold difference in concentration and time. Mice are seen to be asphyxiated within the incapacitating Ct range of these rats by the respiratory pattern analysis model of Matijak-Schaper and Alarie (1982).

Although current animal models have demonstrated a sensitivity for the asphyxiants, they are less sensitive to the irritants. The foot flexion shock/avoidance model (Packham, et al., 1977) was used, for example, to study the sublethal effects of the thermal decomposition products of polyvinyl chloride in the National Bureau of Standards (NBS) fire smoke toxicity test (Levin, et al., 1982). During the thermal decomposition process, hydrogen chloride (HCl) is released. HCl is a potent acid irritant known to cause damage to nasal mucosa as well as to the cornea and cause swelling and closure of the vocal cords which leads to suffocation in man (Flury and Zernik, 1931.) The investigators at NBS were never sure of the occurrence of incapacitation. During the

same exposure, time to incapacitation had a wide range of variation, and some rats actually died before others were incapacitated. In the baboon escape model (Kaplan, et al., 1985) the animals exposed to high concentrations of HCl (up to 17,290 ppm) and acrolein (up to 2780 ppm) were obviously compromised, coughing, gasping, yet were able to "escape" as defined by the model. In general, animals exposed to irritants are close to death when incapacitation occurs. For asphyxiant exposures there is an approximate 2-3 fold difference, both in time of occurrence and concentration, which produces effects between incapacitation and death (Hartzell, et al., 1985; and Kaplan, et al., 1984). This is not true of irritant exposures.

A model dependent on respiratory pattern analysis (Matijak-Schaper and Alarie, 1982; Alarie and Anderson, 1979; ASTM, 1984) was found to be sensitive to many irritants including those found in fire atmospheres; but it is a non-behavioral model. Behavioral models that are sensitive to irritants and likely asphyxiants have been introduced by Wood (1979), Tepper, et al. (1985), and more recently by Malek, et al. (1987). The mouse track escape performance model proposed by Malek, et al., (1987) is not only sensitive to asphyxiating CO and low oxygen atmospheres but also to the irritant HCl. In this model, animals exposed to HCl are fitted with tracheal cannulas prior to testing to eliminate the scrubbing capacity of the mouse nose for HCl and deposit the toxicant more directly into the lower respiratory tract (Anderson and Alarie, 1980). Both of these respiratory modifications are likely to simulate human breathing conditions while running to escape. The incapacitation concentration range of HCl for mice is 951-2150 ppm, within the similar 1000-2000 ppm HCl range reported to be dangerous and possibly lethal for

man within a short period of time (National Academy of Science, 1976). The mouse track model, therefore, demonstrates compromised performance in mice for carbon monoxide and HCl at concentrations and times that have been reported to impair human escape. Aside from this positive feature, this model, in addition to an incapacitation endpoint, contains a "distance traveled/time" sublethal response, which is a behavioral task directly related to the escape process. Impaired escape performance may be observed within two minutes of exposure onset before the occurrence or incapacitation and death. By the distance traveled/time approach, compromised performance can be assessed over a very short period of time at concentrations of toxicants found in fire environments without relying exclusively on an incapacitation endpoint. This is an important contribution to the existing set of animal models, particularly when investigating irritant exposures where the occurrence of an all or nothing incapacitation response has often been questionable, and where the animal is close to death should incapacitation be clearly defined.

Discussions at the fifth meeting of the Japan-United States-Canada cooperative study group on the toxicology of combustion products, March 1986, have advocated a research effort to better define toxicological events influencing escape potential from time of onset of effect through incapacitation to death. A similar recommendation was made by the Committee on Fire Toxicology of the National Research Council in their recent report "Fire and Smoke/ Understanding the Hazards", National Academy Press, 1986.

To elucidate these toxicological events, an approach is needed that measures performance changes which inhibit escape with simultaneously

occurring physiological changes. Although physiologic monitoring of test subjects during and subsequent to drug dosing is common in pharmacologic studies (Craig and Stitzel, 1982), less comprehensive work has been done in the area of toxicity of fire smoke. Studies of animals exposed to fire smoke or gases of fire smoke have primarily been monitored for abnormalities in respiration and blood characteristics. In the University of Pittsburgh combustion toxicity test (Alarie and Anderson, 1981) and other work at the University of Pittsburgh (Barrow, et al., 1976; 1977, 1978; Alarie, et al., 1980; Matijak-Schaper and Alarie, 1982), continuous respiratory pattern analysis of mice have been made. In these studies evidence of irritation, asphyxiation and death may be observed during exposure and recovery periods. Such analyses were accomplished by the use of a body plethysmograph in which pressure changes were measured during spontaneous respiration by mice.

The pulmonary function of guinea pigs following exposure to smoke from different materials was evaluated by a CO_2 challenge method developed by Wong and Alarie (1982). In this method, normal guinea pigs respond to 10% CO_2 by increasing their breathing frequency by approximately 1.5 times and tidal volume by 3 times. The inability to respond to the CO_2 challenge in this manner, by animals exposed to the thermal decomposition products of PVC (Wong et al., 1983) and wood (Wong, et al., 1984) and pure HCl gas (Burleigh-Flayer, et al., 1985) reflects evidence of pulmonary dysfunction. In these studies a whole body plethysmograph was used.

Physiologic changes that occur upon exposure to combustion atmospheres and gases have also been observed by arterial blood sampling and assay. Routine measurements of COHb, pH, PO_2 and PCO_2 were made

from cannulated rats during the development of the NBS smoke toxicity test (Levin, et al., 1982). Studies of this nature, using the cannulation technique and incapacitation model of Packham, have been done at the University of Utah (Packham, et al., 1977), Southwest Research Institute (Kaplan, et al., 1984) and Olin Corporation (Condit, et al., 1978). The primary purpose of these studies was to determine the level of COHb in the blood at the time of behavioral incapacitation.

Incapacitation in rats was also associated with the physiologic onset of cardiac arrhythmias upon exposure to carbon monoxide. This relationship was studied by Gaume, et al. (1981) at the McDonnell Douglas Corporation. These electrocardiogram recordings, as well as the measurements of blood and pulmonary function described above, were taken from quiescent animals.

For the assessment of escape potential from fire smoke, levels of toxicity and physiologic events should be determined from active animals. Parent, et al., (1979b) while using the pole-climb conditioned avoidance/escape behavioral model, saw substantially lower LC_{50} values for thermal decomposition products' exposures of various synthetic foams than previously reported in sedentary animals (Parent, et al., 1979a). The investigators suggested that the enhanced toxicity (lower LC_{50}) was due to the increased respiration and metabolism of the actively climbing rats, and consequently the dose of smoke received. Hilado and Cumming (1977) studied the toxicity of various gases including carbon monoxide on rats and mice, and noted a direct relationship between their metabolic rate (oxygen consumption) and toxicity. These investigators feel as did Saito (1977) after studying carbon monoxide toxicity in active mice, that an enhanced respiratory minute volume coincident with

an elevated metabolism resulted in higher toxicant loading and the higher observed toxicity.

The maximal oxygen consumption ($\dot{V}O_{2\ max}$) measurement has been used clinically to evaluate the physiologic capacity of an individual to adapt to the increased metabolic needs of work or exercise. This ability is determined by many factors, such as ventilation of the lung, pulmonary diffusion, O_2 and CO_2 transport of the blood, cardiac function, capillarity and oxidative capacity of the musculature (deVries, 1980). If any of these systems are compromised, the $\dot{V}O_{2\ max}$ would decline. Specifically, human studies have shown a reduction in $\dot{V}O_{2\ max}$ to be directly related to blood carboxyhemoglobin which would impair O_2 transport (Pirnay, et al., 1971; Vogel, 1972a, b). Other than these studies with relatively low concentrations of CO, the $\dot{V}O_{2\ max}$ or a submaximal oxygen consumption index has not been used to evaluate human performance during exposures to gases known to be in fire atmospheres. This is understandable considering the obvious problems associated with the use of humans in toxicologic studies. No animal data, however, exist either. Such an approach with animals would provide a physiologic mechanism to evaluate the potential to perform work (escape) that can be related to man.

D. Proposed New Approaches

In view of this deficit in available animal behavioral models and the realization that an escape predictive sublethal response observed in exposed active animals is required to effectively evaluate performance, the guinea pig ergometer model is now proposed. Although the mouse has been shown to be sensitive to both asphyxiants and irritants (Wood, 1979, Matijak-Alarie, 1982; ASTM, 1984; Malek, et al., 1987), it is a

small animal and particularly when it is active physiologic measurements are difficult to obtain. The guinea pig, on the other hand, is a much larger animal that could easily provide samples of blood for analysis and be adaptable to physiologic measurements.

Evidence exists that humans are incapacitated at relatively low levels (1000-2000 ppm) of HCl in short periods of time (National Academy of Sciences, 1976). The mechanism suggested for these incapacitations is suffocation due to constriction of the larynx and pulmonary bronchi.

A good animal for the investigation of this mechanism is the guinea pig due to its wide distribution and high reactivity of smooth muscle throughout their respiratory tract. In addition, the guinea pig demonstrates a good ventilatory response to CO_2 (Wong and Alarie, 1982) a feature that has proven to increase the sensitivity of a guinea pig model (Schaper, et al., 1985). Neither a mouse or a rat has a wide distribution of respiratory smooth muscle (Lucia and Alarie, unpublished data) nor do they respond well to CO_2 challenge (Burleigh-Flayer, et al., 1985).

The aforementioned characteristics of the guinea pig make it a promising animal model for continued fire smoke toxicity studies. Therefore, it has been adopted for experimental work on performance (escape) impairment, the basis of this thesis.

The guinea pig, unlike the mouse which is naturally curious and likes to run (Malek, et al., 1987), had to be enticed to run while exposed to the toxicant of interest. Therefore, the first objective of this study was to design and construct a variable speed ergometer for

the guinea pig where it remained enclosed in a ventilated gas tight exposure box.

As a second study objective, an experimental protocol was to investigate the inhalation toxicity of a prototype asphyxiant (carbon monoxide) and irritant (hydrogen chloride) on a running guinea pig. The effects of toxicity and therefore the performance of the guinea pig were to be examined both behaviorally and physiologically. On a behavioral basis, incapacitation as an escape predictive endpoint would be defined. Similar to the type of data generated in the mouse track model by knowing the speed of the running guinea pig and the time of incapacitation, the distance traveled by the guinea pig could be calculated. Physiologic performance would be evaluated by $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$. Oxygen consumption is an index of an animal's overall metabolism and energy expenditure during movement such as that required to escape a fire.

The third and final objective of this work is to suggest how the behavioral and physiological data obtained from the guinea pig ergometer model may be used to predict human performance while exposed to similar toxic environments. Prior to developing the guinea pig model, the mouse track model referred to above was developed.

METHODS AND MATERIALS

A. Chemical Toxicants

Matheson analyzed 9.84% carbon monoxide gas, balance nitrogen or pure CO, and .0975% HCl, balance air were used to produce toxic environments for guinea pigs exposed either while sedentary or while exercising. A Miran infared analyzer (model 1A) and the Ostekridischen-Stickstofferwerke method (Leithe, 1971) were employed for the analysis of carbon monoxide and hydrogen chloride respectively. These methods and calibration procedures are described in Appendix A.

B. Animal Source and Pre-exposure Handling

English short haired male guinea pigs (cavia porcellus) ranging from 300-325 grams were purchased from Hilltop Lab Animals Inc. Scottdale, PA. They were housed four to a cage and fed water and guinea pig chow (Ralston Purina, Co., St. Louis, MO.) ad libitum with a 12 hour light/dark cycle. Guinea pigs remained in their cages prior to sedentary exposures.

Those animals considered for running exposures began an exercise training program. Guinea pigs that showed a distinct willingness and ability to run continued to be trained (to be described). All animals were weighed regularly and ranged from 355-385 grams at the time of exposure.

C. Guinea Pig Ergometer/Exposure Chamber

Schematic drawings including the dimensions of the guinea pig ergometer are shown in Figures 1, 2, and 3. A 4.9 L keystone shaped plexiglas exposure chamber completely encloses a stainless steel wheel (diameter 454 mm). When gas was introduced at any point in the system it would surround the wheel and equilibrate with the entire chamber volume. Six minutes were required to reach 95% equilibration (see Appendix B for this calculation and calculation of ergometer volume). The cylinder of the wheel (95 mm wide) was covered by a 5 mm silicone rubber tread. The tread provided a functional surface onto which the guinea pig ran (during running exposures) while confined within the upper wedge-shaped section serving as an exposure chamber. The running area was 73 mm wide and 270 mm long. Bumper brushes were located at the front and back of the running area which served to encourage the guinea pigs to run. Those in the front were attractive to the guinea pig as evidenced by their desire to chew on them, while those at the back were a negative stimulus to the animal's hind end. Other apparatus (i.e., electrical shock) used to motivate rodents to run (Bernard, et al., 1971, Pitts, et al., 1971) were not used. Inlet and exhaust ports were also located at the front and the back while multipurpose ports, were arranged along the top arc of the exposure chamber.

The guinea pig was introduced into and removed from the system by a circular rubber stoppered port on the face of the chamber.

The entire plexiglas front plate can also be removed by disengaging a series of 36 wing nuts along its perimeter for cleaning the inside of the apparatus. A rubber gasket between the side rim of the exposure chamber and front plate served to effectively seal the system when the

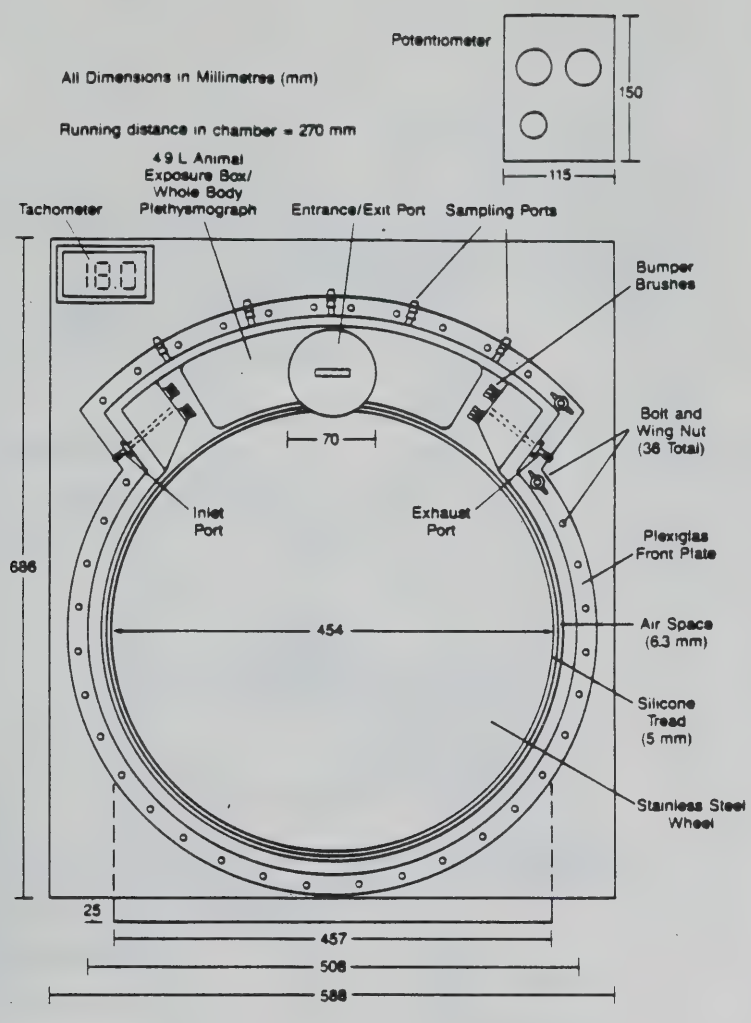


Figure 1. Guinea pig ergometer/exposure chamber used for exposure to air (control), CO or HCl during exercise. Guinea pigs ran at speeds ranging from 0 to 18 rpm (0.0 to 1.59 km/h), on top of a motor driven wheel, enclosed in a keystone shaped plexiglas chamber. The guinea pig is introduced and removed from the wedged running area of the exposure chamber by a rubber stopper on the chamber's front plate. To reduce the air volume of the exposure system, stainless steel plates were welded to the inside rim of the stainless steel wheel, both front and back. This resulted in an air volume of 4.9L for the exposure system and is necessary to insure that the small pressure change (ΔP) due to breathing can be recorded and for rapid mixing of introduced toxicants.

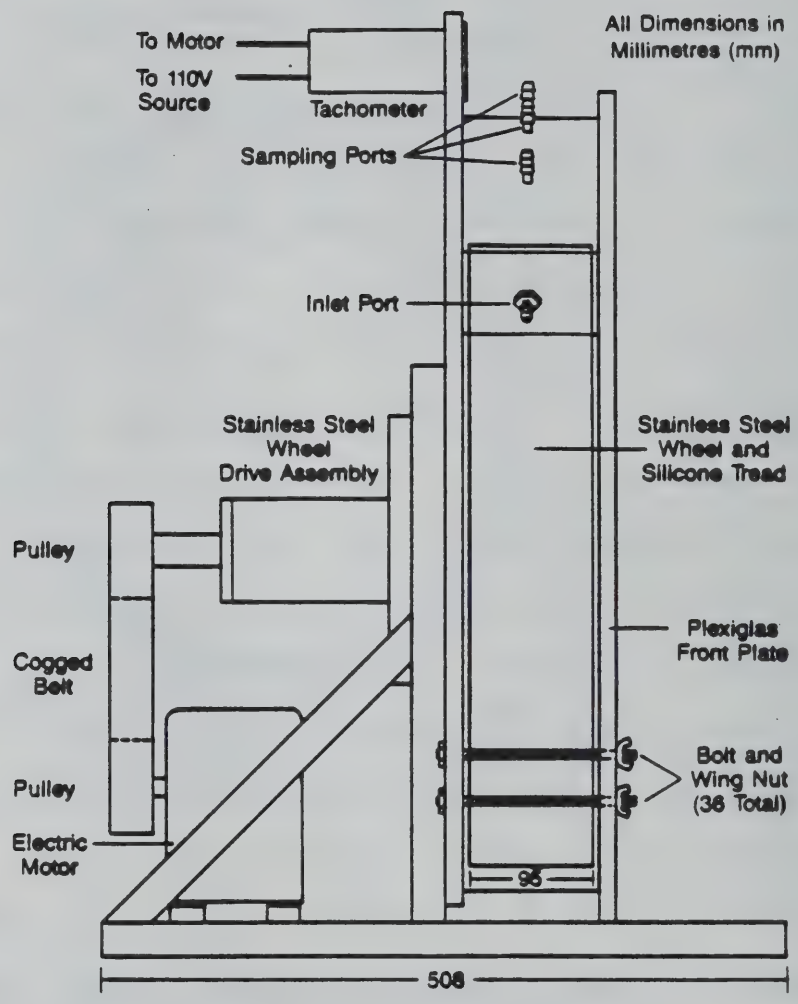


Figure 2. Side view of the guinea pig ergometer/exposure chamber

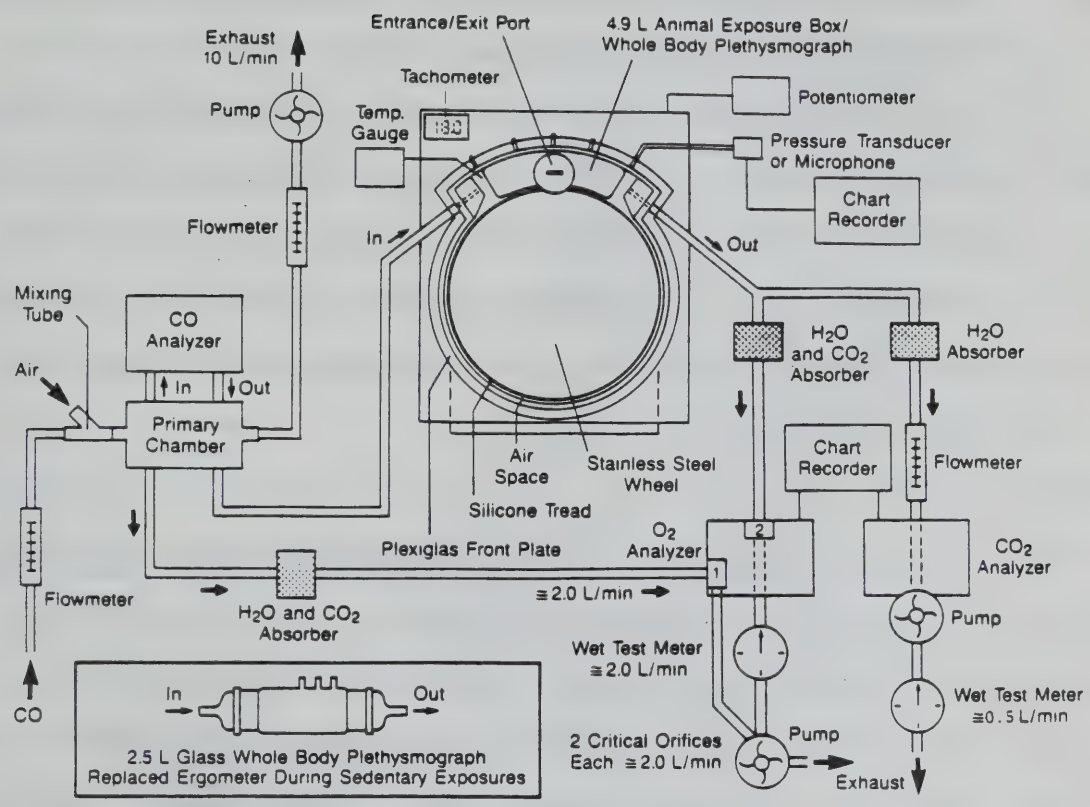


Figure 3. Guinea pig ergometer/exposure system used for air control and CO exposures during exercise. The enclosed ventilated ergometer functions as an exposure chamber and whole body plethysmograph. Tidal volume (ΔP) and f are continuously monitored, along with $\dot{V}O_2$ and $\dot{V}CO_2$. Desired concentrations of CO are prepared in the mixing tube by the appropriate combination of metered 100% CO and air. A glass exposure chamber replaced the ergometer during sedentary exposures.

wing nuts were gently tightened by hand. In addition an O-ring oil seal surrounded the wheel's axle as it passed through the back wall of the chamber to engage with the gear/motor assembly and prevented leakage of gases flowing through the system.

The rubberized wheel was driven by a variable speed two directional D.C. motor (Bodine Electric, Chicago, IL) at speeds ranging from 0 to 18 rounds/min (rpm) or 0.0-1.59 kilometers/hour (km/h). The speed was controlled by a dual range potentiometer (Bodine Electric, ASH-401) and was digitally indicated on a tachometer (Visi-tach, VT-3, Minarik Electric, Los Angeles, CA).

D. Exposure System for CO and Measurement of Physiologic Events During Exercise and Sedentary Exposures

The exposure and physiologic measurement systems that were used for CO exposures during exercise are diagramed in Figure 3. The system design for sedentary exposures was the same except a 2.5 L glass whole body plethysmograph replaced the guinea pig ergometer. Since the exposure chamber completely encloses the wheel yet part of a flow-through system, the exposure box functions as a whole body plethysmograph, thus making the continuous measurement of respiratory events (f and ΔP) possible. This approach is based on the method of Wong and Alarie (1982) where unrestrained guinea pigs could be monitored for extensive periods of time while exposed to air or other gas mixtures. Pressure changes (ΔP) that occur in the box as a function of the animal breathing are detected by a sensitive microphone (Fukuda TY 303, Gould Electronics, Cleveland, OH) that is attached to one of the sampling ports. Upon inspiration a normal guinea pig (350 grams)

inspires about 1.5 ml of air from the chamber; this air is both heated to body temperature (37.5°C) and humidified in the lungs thus increasing the chamber pressure. The reverse occurs during expiration. These pressure changes are proportional to tidal volume (Wong and Alarie, 1982). By counting the number of pressure changes per unit time respiratory frequency could be obtained. Respiratory events were monitored on a chart recorder (Western Graphtec, Mark VII, Irvine, CA). As shown in Figure 3, the exposure chamber is air tight but air is continuously flowing through inlet and outlet ports. To record the pressure changes (ΔP) created during each breath, a long tube is attached to both the inlet and outlet ports to create sufficient resistance to in effect seal the system for recording ΔP. The length of tubing used was 100 mm and the inside diameter was 3 mm. To test that the system was working properly a pump was used to simulate breathing by the animal. By pumping known volumes of air at frequencies of 60 to 300/min the inlet and outlet tubing were adjusted so that ΔP created in the exposure system was the same when the system was completely closed as while air was flowing through it.

A glass mixing tube and primary chamber were located in the front of the system. Here appropriate proportions of air and toxicant were combined to produce desired exposure concentrations. Carbon monoxide was continuously monitored from the primary chamber. When the entire system was in operation a total of 12.5 L of gas was passed through the primary chamber. 10 L/min were immediately exhausted and 2.5 L/min were pulled through the exposure chamber by pumps located either within (at 0.5 L/min) or beyond (at 2.0 L/min) the CO_2 analyzer (Sensormedics LB-2) and two-cell differential oxygen (Servomex dual cell) analyzer

respectively. The CO_2 analyzer was calibrated with ambient air (.03% CO_2) and standard gases of 1.0% and 5.0% CO_2 obtained from Radiometer America, Inc. The CO_2 monitored from the outlet of the exposure box was taken to be the % CO_2 output since .03% was assumed to be 0.0%. The test atmosphere was passed through a desiccant and H_2O was removed prior to CO_2 analysis. Both H_2O and CO_2 were scrubbed through a desiccant/sodium hydroxide-silicate filter prior to O_2 analysis. Oxygen was also sampled before the exposure chamber (pre-chamber) at the point of the primary chamber. The difference in pre-chamber O_2 concentration and post-exposure chamber was recorded as the O_2 taken up by the guinea pig. The O_2 analyzer was calibrated with zero O_2 (pure nitrogen), 10.9% O_2 and room air (20.90% O_2). Both O_2 uptake and CO_2 output were continuously recorded on a Graphtec Servocorder. After analysis the gas samples were exhausted to a hood.

A temperature gage and flexible probe (Baily, model BAT 8) monitored the exposure chamber temperature. In addition, the temperature of the room and barometric pressure were measured prior to each experiment. These measurements were used in the subsequent calculation of O_2 uptake (ml/kg/min) and CO_2 output (ml/kg/min) at standard temperature and pressure, dry (STPD).

E. Exposure System for HCl Exposures During Exercise

Since HCl is a corrosive acid, no physiologic measurements were made during exposure to prevent damage to the analytical instrumentation. The system for exposure to HCl during exercise is illustrated in Figure 4. Pressurized gas was introduced through the exposure chamber at 10 L/min. The animal exposure concentrations were

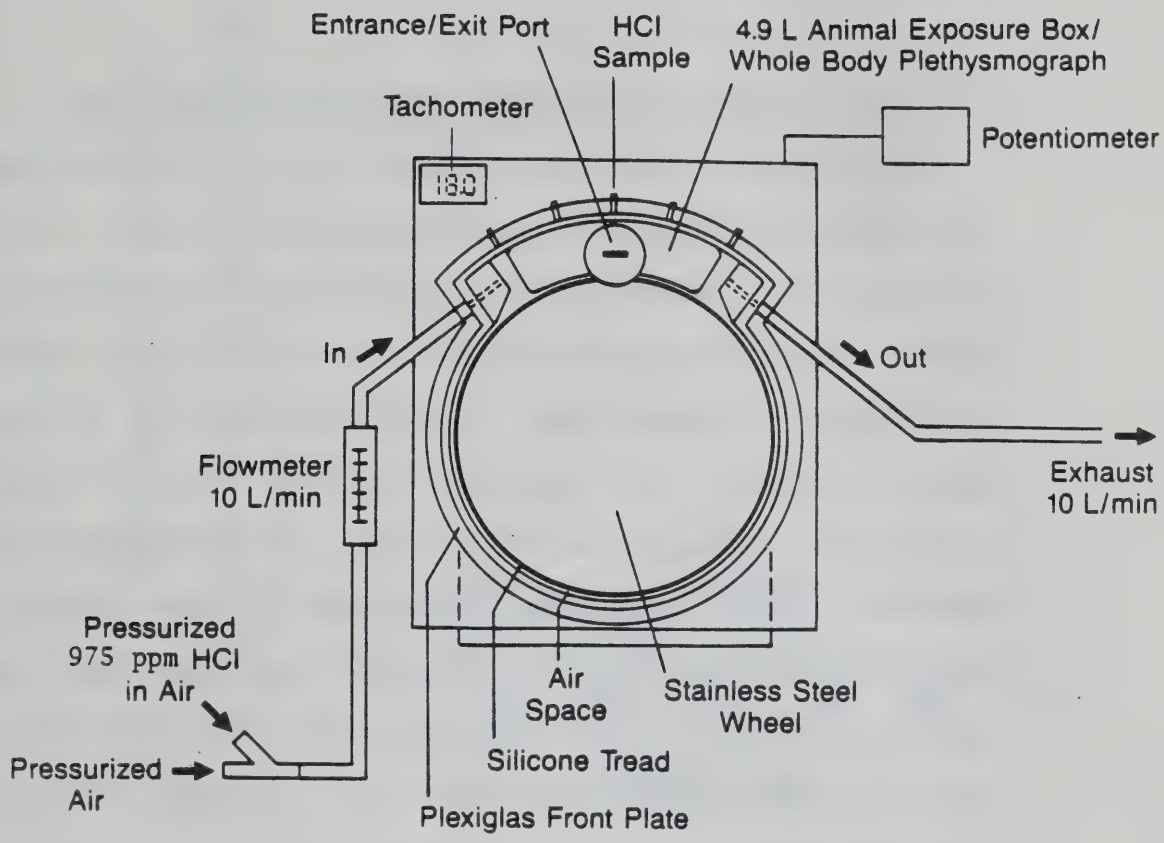


Figure 4. Exposure system for HCl exposures during exercise. Either pressurized air or HCl (975 ppm in air) were introduced to the chamber at 10L/min. HCl was sampled from the top chamber port for subsequent chemical analysis. No physiologic measurements were made during these exposures due to the corrosive nature of HCl gas.

determined by sampling HCl from the top of the exposure chamber at a rate of .95 L/min into two miget impingers in series containing 0.1 N NaOH.

F. Animal Training for CO and HCl Exposures During Exercise

Twelve guinea pigs typically made up each starting group. The first two days involved familarizing the guinea pigs to the ergometer environment and determining which animals had the potential to be runners. Those with potential were trained daily for a few days then were trained on alternate days. The protocol outlined in Figure 5 was closely followed. The speed and running time for each animal progressively increased as the guinea pig developed physiologic endurance. It was considered trained when it could complete the 55-minute running protocol in air. Usually 7 to 10 days were required to train a guinea pig to run. Only 30 to 50% (4 to 6) of the original group of 12 guinea pigs were able to be trained. This rate was consistent with that described in earlier studies in which guinea pigs were similarly trained and oxygen consumption was measured (Pasquis, et al., 1970). This protocol was designed so that a warm up period during the first 4 minutes was followed by a speed (1.59 km/h) that would increase metabolic activity (as indicated by O_2 uptake and CO_2 output) by a factor of 2 to 3 times baseline. This speed was maintained for 36 minutes so that a 30 minute exposure to a toxicant could occur and could be compared to other exposures where guinea pigs were similarly exposed for 30 minutes under sedentary conditions in this study or in previous work (Burleigh-Flayer, et al., 1985).

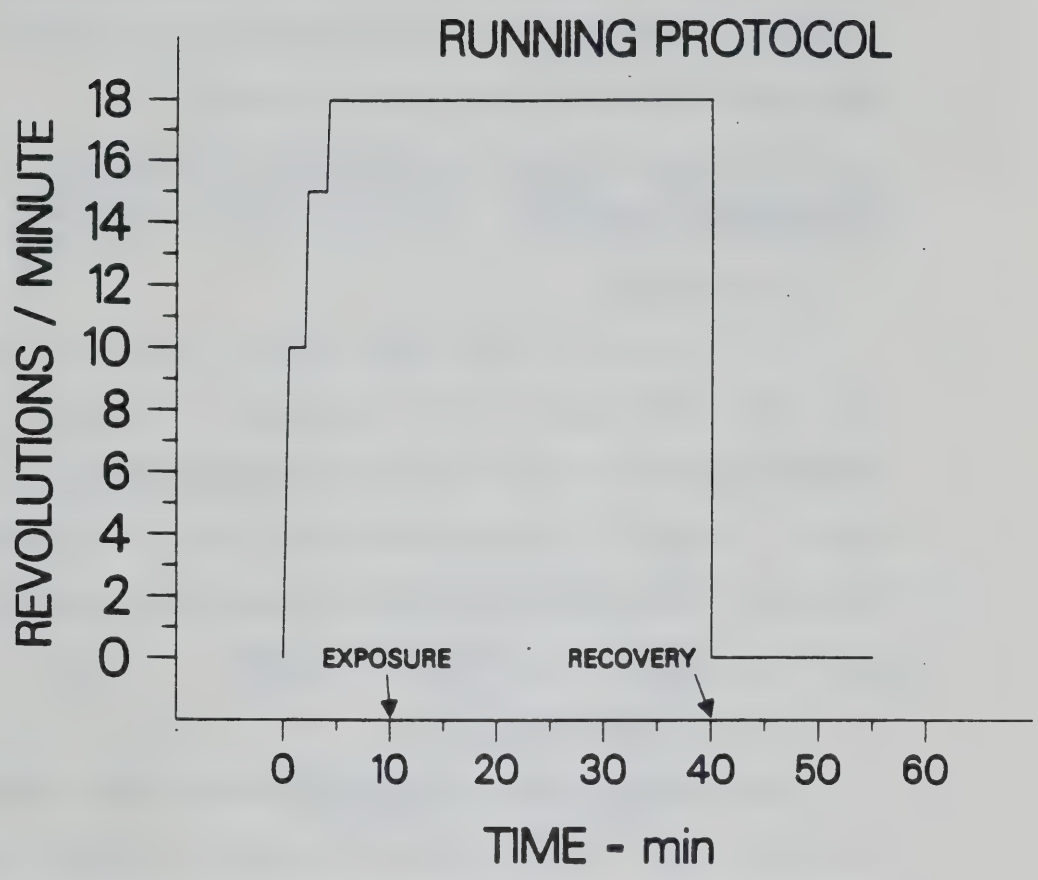


Figure 5. A guinea pig was considered trained to run when it could complete this 55-minute running protocol. This protocol was followed for air control, CO and HCl exposures during exercise. See text for explanation of protocol and deviations from it during specific exposures. The speeds, 10 rpm, 15 rpm and 18 rpm are equivalent to 0.88, 1.32, and 1.59 km/h, respectively which correspond to 0.54, 0.82 and 1 mile/hour.

At minute-0 the ergometer speed was quickly brought to 10 rpm for 2 min, increased to 15 rpm for 2 min, then to 18 rpm for an additional 36 minutes. At protocol minute-40 the wheel was turned off, and the guinea pigs were allowed to recover for 15 minutes.

G. Sedentary Exposures

1. Air Control

In this series of experiments guinea pigs were simply placed in a whole body plethysmograph during exposure. Eight control animals were exposed to air for 30 minutes followed by a 15 minute air recovery period. Physiologic measurements were taken at baseline as well as continuously during the designated exposure and recovery periods. The sedentary protocol is outlined in Figure 6.

2. CO - Normal Guinea Pigs

After baseline physiologic measurements were taken in air, six groups of 4 guinea pigs each were exposed to single concentrations of carbon monoxide for a total of 30 minutes followed by a 15 minute air recovery period unless an animal died. These exposure concentrations were 19,000, 17,500, 16,000, 14,500, 8,700, or 5,700 ppm of CO. Physiologic measurements were continuous. Since the guinea pig has a high center of gravity they fall to their sides upon narcosis and asphyxiation. This behavioral endpoint was used to represent incapacitation. This type of incapacitation is termed **S**-incapacitation (sedentary incapacitation) resulting from sedentary exposures, and is differentiated from **E**-incapacitation (exercise incapacitation) as observed during exposure to CO while running.

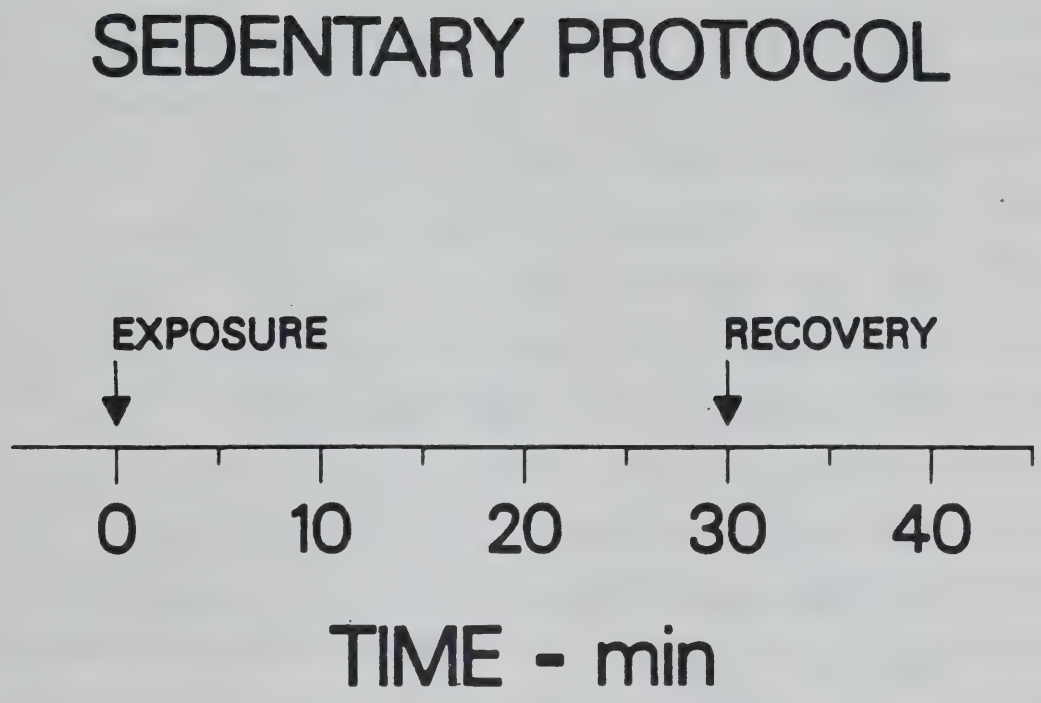


Figure 6. Sedentary guinea pigs were exposed to CO according to this protocol. Unless an animal died, the guinea pig was exposed to the toxicant for 30 minutes followed by a 15 minute recovery in air. Control animals were exposed to air for 45 minutes. All animals simply sat in a glass whole body plethysmograph during exposures.

3. CO - Cannulated Guinea Pigs

Sixteen guinea pigs in this set of experiments were fitted with carotid artery cannulas at least 24 hours prior to exposure to carbon monoxide. A brief description of the procedure is found in Appendix D. Four groups of 4 guinea pigs each were exposed to 19,000, 17,500, 16,000 or 14,500 ppm of carbon monoxide for 30 minutes followed by a 15 minute air recovery unless an animal died. These experiments were designed to reproduce the carbon monoxide exposures of normal guinea pigs just described except for surgically placement of the carotid artery cannula used to draw 1 ml blood samples at **S**-Incapacitation for carboxyhemoglobin (% COHb) measurements. Blood samples were alternatively taken by cardiac puncture if death occurred. Both the times (min) of incapacitation and death were recorded. Development of a spectrophotometric method for % COHb measurement in the guinea pig is described in Appendix E.

Physiologic measurements (f, ΔP, O_2 uptake, and CO_2 output) were taken from these guinea pigs only at baseline and compared to normal guinea pigs at baseline to determine if they were compromised by surgery and therefore suitable for carbon monoxide exposures. Any statistically significant differences among the baseline parameters between the two groups were determined by the use of a student t test at a level of significance, $p < 0.05$ (Armitage, 1971).

H. Exposures During Exercise

1. Air Control

The 55-minute running protocol (Figure 5) was followed exactly during air control exposures. The aforementioned physiologic measurments (f, Δ P, O_2 uptake, CO_2 output) were taken at baseline,

throughout the run as well as during the recovery period for 8 guinea pigs exposed only to air.

2. CO

The protocol (Figure 5) was similarly followed during carbon monoxide exposures, however the toxicant was introduced at protocol minute-10. Running guinea pigs reached a stable O_2 uptake level by minute-10 and it was viewed to be an appropriate time to initiate the exposure. Two groups of 4 guinea pigs each were exposed to carbon monoxide during exercise. The exposure concentrations were 2,200 and 8,290 ppm CO. The guinea pig would continue to run until the wheel was stopped at the scheduled minute-40 or when it became incapacited, in which case the wheel was also stopped. The time of incapacitation was recorded. Regardless of the time of incapacitation all test animals were exposed to CO for a total of 30 minutes. Incapacitation that occurred during exercise exposures or E-incapacitation was defined as that point when a trained guinea pig collapsed and could no longer run. This event was confirmed by restarting the wheel several times in the minutes immediately following the called E-incapacitation and determining that the animal truly could not run. Other behavioral effects observed in the guinea pig such as "slipping" of front paws and "sitting" at the back of the chamber were also noted prior to incapacitation as well as a decrease in $\dot{V}O_2$.

3. HCl

During the first 10 minutes of the running protocol, pressurized air was introduced at the Y-tube at 10 L/min. At protocol minute-10 the air was turned off and 0.0975% (975 ppm) HCl in air was alternatively introduced at 10 L/min. During each 6 minute exposure the wheel was

turned off at E-incapacitation. One 3 minute sample for analysis of HCl was obtained in each experiment. Five trained guinea pigs were exposed at 411, 530, 572, 591 or 652 ppm of hydrogen chloride, respectively.

At the end of each exposure the guinea pigs were sacrificed if not already dead as a result of the exposure. Their larynx and lungs were removed and examined for gross abnormalities, then the lungs were fixed by infusion of 10% buffered Formalin and placed in a jar containing the same along with the larynx. Lung weights (grams) before and after infusion as well as the infusion volume (ml) were recorded. Although these tissues were fixed for subsequent histopathologic examination, pathologic findings will not be available for discussion in this thesis. No experiment was conducted with HCl in sedentary conditions since these data were previously obtained by Burleigh-Flayer et al. (1985).

I. Data Analysis

1. Respiratory Rate (f)

Unless the chart deflections were unreadable due to body movements, the breaths in the last 15 sec of every other recorded minute were counted and multiplied by 4 to give breaths/min. An average value obtained over three minutes at baseline was also calculated.

2. Tidal Volume (ΔP),

With each animal quiet in the exposure chamber (used as as a whole body plethysmograph) a pump was used to supply a known volume displacement (4 ml), coincident pressure change caused a deflection on a chart recorder. This information was used to calculate a calibration factor that was applied to all subsequent chart deflections produced by the guinea pig. A 4 ml volume produced by the pump resulted in a 25

division deflection on the chart recorder at a sensitity of 500X and the guinea pig deflected (on the average) the pen 13 divisions at a chart sensitivity of 100X. The calibration factor and ΔP were calculated as follows:

$$\frac{\text{4 ml pump displacement}}{\text{No. division deflection by pump}} \times \frac{\text{chart sensitivity guinea pig}}{\text{chart sensitivity for pump}} = \text{Calibration (ml/div) factor}$$

$$\frac{4 \text{ ml}}{25 \text{ div}} \times \frac{100}{500} = .0320 \text{ (ml/div)}$$

Breath (divisions) X Calibration Factor (ml/div) = Tidal Volume (ml)

8 (Divisions) X .0320 (ml/div) = .2560 (ml)

The tidal volume was calculated by taking the average divisions deflected in the same breaths that were counted (for f) during the last 15 seconds of every other minute recorded. An average baseline value was also determined. Therefore tidal volume is considered a measurement made indirectly from pressure changes (ΔP) recorded from the exposure system. Such measurements were calibrated in ml but the ml values were not the absolute volume of inspired air with each breath.

3. O_2 Uptake ($\dot{V}O_2$)

The difference in oxygen between pre and post-exposure chamber, were extracted from the chart record at baseline and then every other minute starting with minute 3 of each exposure protocol. O_2 uptake (ml/kg/min) STPD was calculated for each of these minutes.

O_2 uptake (ml/kg/min, STPD) = A x B x C x D x E where,

A = % O_2 difference between pre and post chamber

B = Chamber air flow (ml/min)

$$C = \frac{\text{Barometric pressure (mmHg)}}{760 \text{ (mmHg)}}$$

$$D = \frac{270\ ^{o}K}{\text{Room temperature } (^{o}K)}$$

$$E = \frac{1}{\text{Guinea pig body mass (kg)}}$$

4. CO_2 Output ($\dot{V}CO_2$)

Carbon dioxide values were extracted from the chart record at baseline and alternate minutes of the protocol starting from minute-1. The CO_2 output (ml/kg/min) STPD was calculated for each of these minutes by the equation outlined for O_2 uptake except the CO_2 value was substituted for the O_2 difference value.

For each parameter (f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$) the mean values ($\pm$ SD, % C.V.) at baseline and for each designated minute of the protocol were calculated for 8 air control animals and groups of 4 animals exposed to each of the exposure concentrations of carbon monoxide. After the mean for each minute was calculated, each value was then divided by the baseline value to normalize the data. The 4 guinea pigs that were

exposed to 2,200 ppm carbon monoxide while running became incapacitated, and the wheel was stopped over a wide range of time (from 8.5 min to 15.5 min) and therefore the physiologic events at each protocol minute were not meaned. The data of each animal were treated individually and normalized by the average baseline values of four animals.

5. Distance Traveled During Running Exposures

Since the guinea pig is running at 1.59 km/h or .0265 km/min, and travel time was determined by E-incapacitation the distance traveled was calculated as shown below.

.0265 km/min	X	Mean time to incapacitation (min)	=	Distance traveled (km)

RESULTS

A. Post Arrival Weight Gain in Running vs. Non-running Guinea Pigs

The weight profile of running (n = 15) vs non-running (n = 15) guinea pigs is shown in Figure 7. Average values for alternate post-exposure days (where the coefficients of variation were under 9%) are expressed as ratios. The values used to normalize the runner and non-runner data were 304.8 $\pm$ 20.8 and 306.4 $\pm$ 22.6 grams respectively. A t test (Armitage, 1971) showed there to be no statistically significant differences between the two groups at any time.

B. Sedentary Exposures

1. CO and Air Control Exposures - Normal Guinea Pigs

Respiratory rate (f), tidal volume (ΔP), oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) measurements for air control and six exposure concentrations of carbon monoxide (5,700, 8,700, 14,500, 16,000, 17,500, 19,000 ppm) are presented in Figures 8 through 13. The average air control values (n = 8) for each physiologic parameter were expressed as ratios normalized by their respective baseline value listed in Table 3. By this approach the average pre-exposure value at protocol minute-0 was set to 1.00. The coefficients of variation for air control f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ mean values were less than 26, 35, 30 and 32% respectively. These guinea pigs were often active, free to walk and explore within body plethysmograph, and this is likely to account for

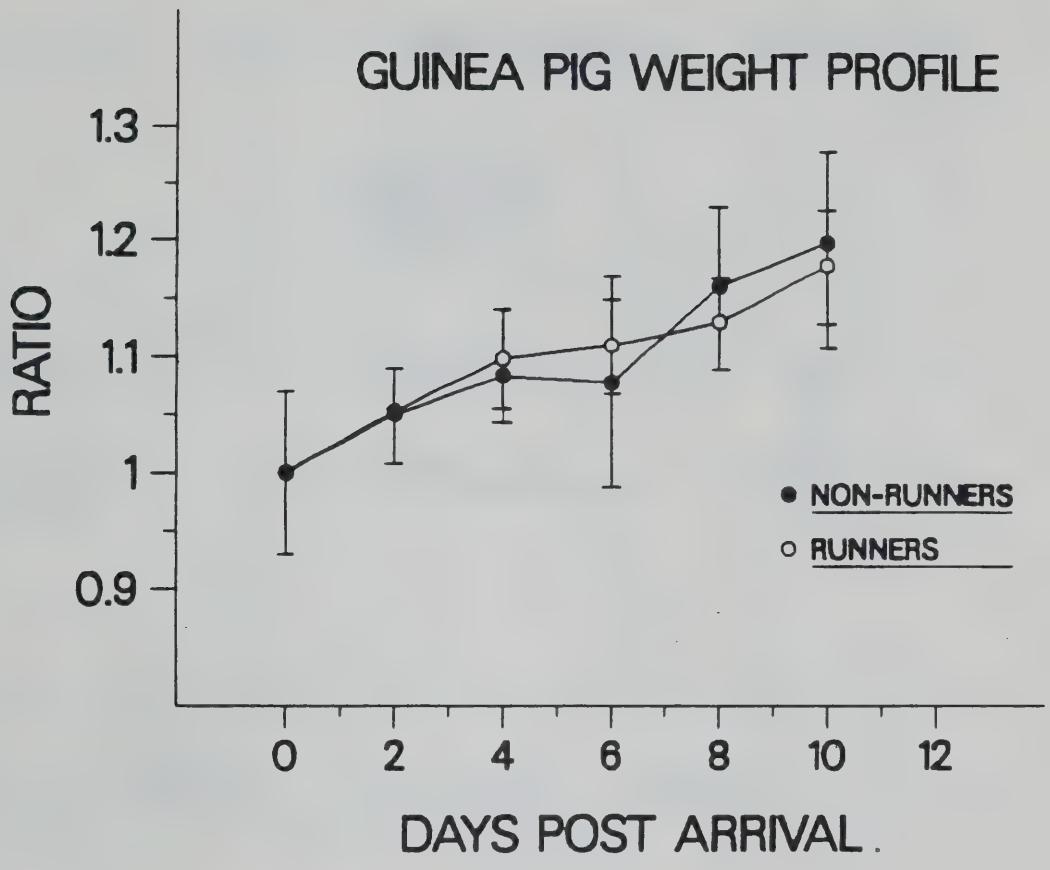


Figure 7. Weight profile of guinea pigs that were sedentary (non-runners, n = 15) or that were trained to run (runners, n = 15) in the 10 days following their arrival. The neon $\pm$ SD weights were normalized by the average weight of each group of guinea pigs respectively on the day of arrival and expressed as ratios. The coefficient of variation was less than 10% at all times. No statistical difference was found between the two groups.

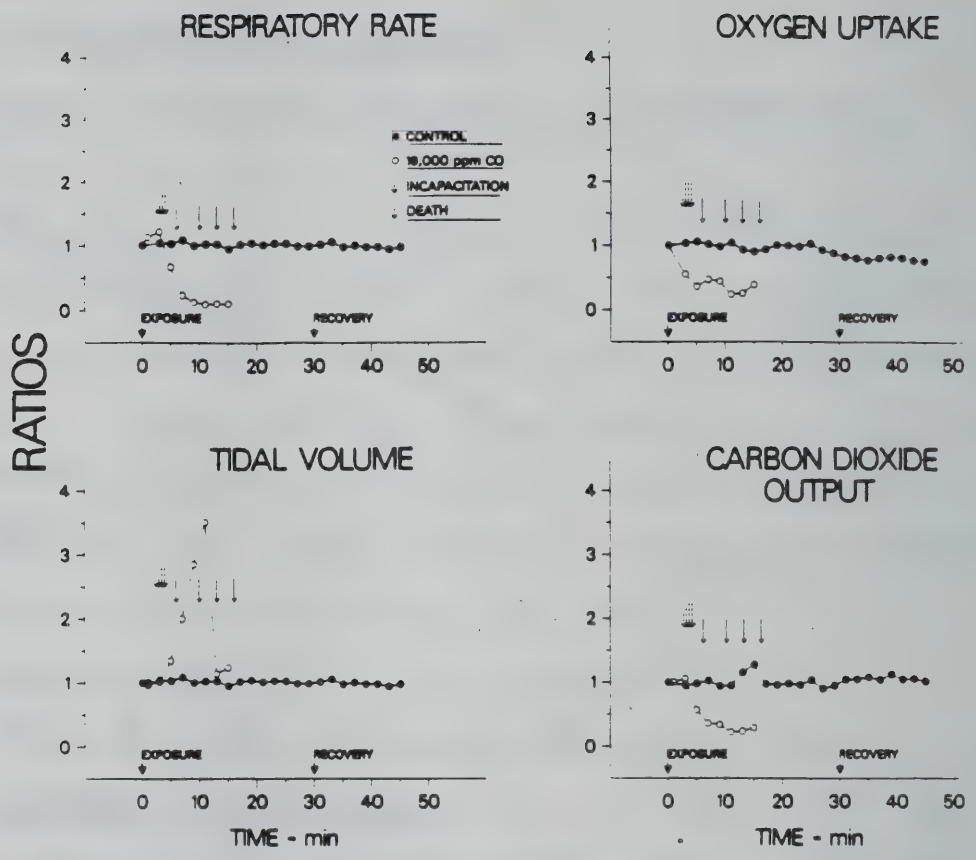


Figure 8. Physiologic measurements, f, ΔP, $\dot{V}O_2$, and $\dot{V}CO_2$ of guinea pigs during 30 min sedentary air control (n = 4) and 19,000 ppm CO (n = 4) exposures followed by 15 min air recovery. All animals were S-incapacitated and died before 16 min. The data (means) of each group were normalized by their respective average values (Table 3) and expressed as ratios. The % CV were less than 35% for control and less than 30% of the mean values for CO exposed animals for all physiologic parameters.

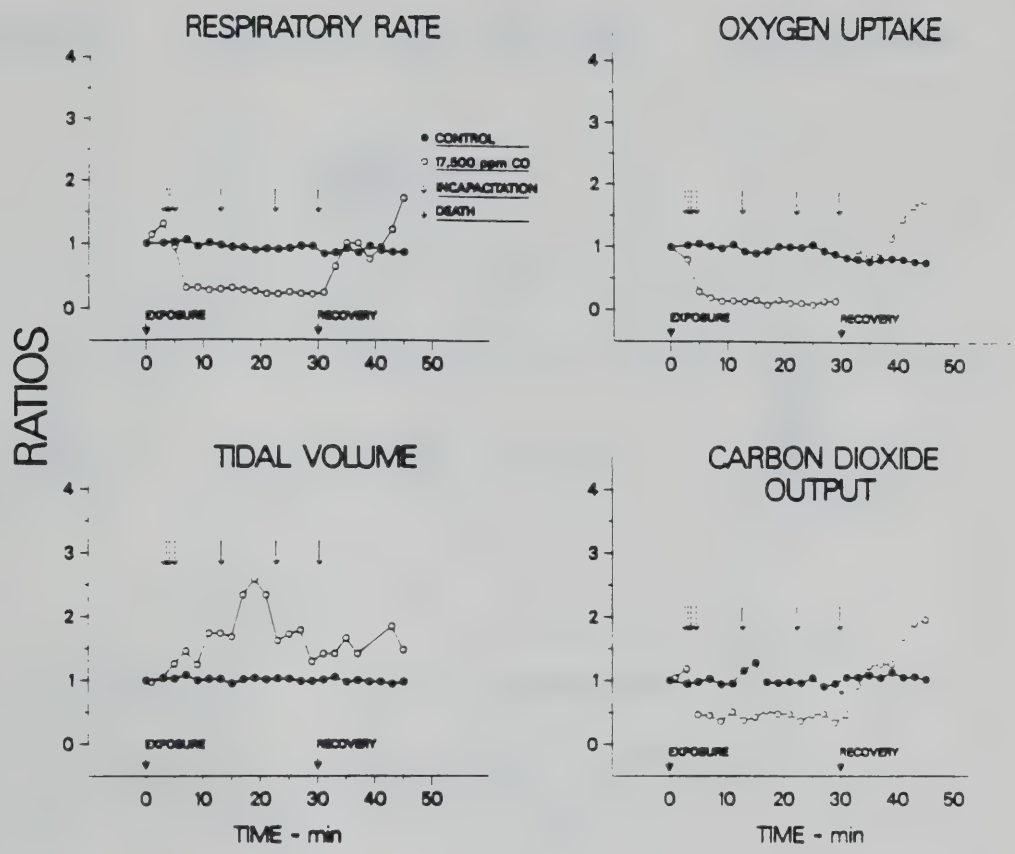


Figure 9. Physiologic measurements, f, ΔP, $\dot{V}O_2$, and $\dot{V}CO_2$ of guinea pigs during 30 min sedentary air (n = 4) and 17,500 ppm CO (n=4) exposures followed by 15min air recovery. All animals were S-incapacitation and three died. The data (means) of each group were normalized by their respective average values (Table 3) and expressed as rations. The % CV were less than 35% for control and less than 30% of the means for CO exposed animals for all physiologic parameters.

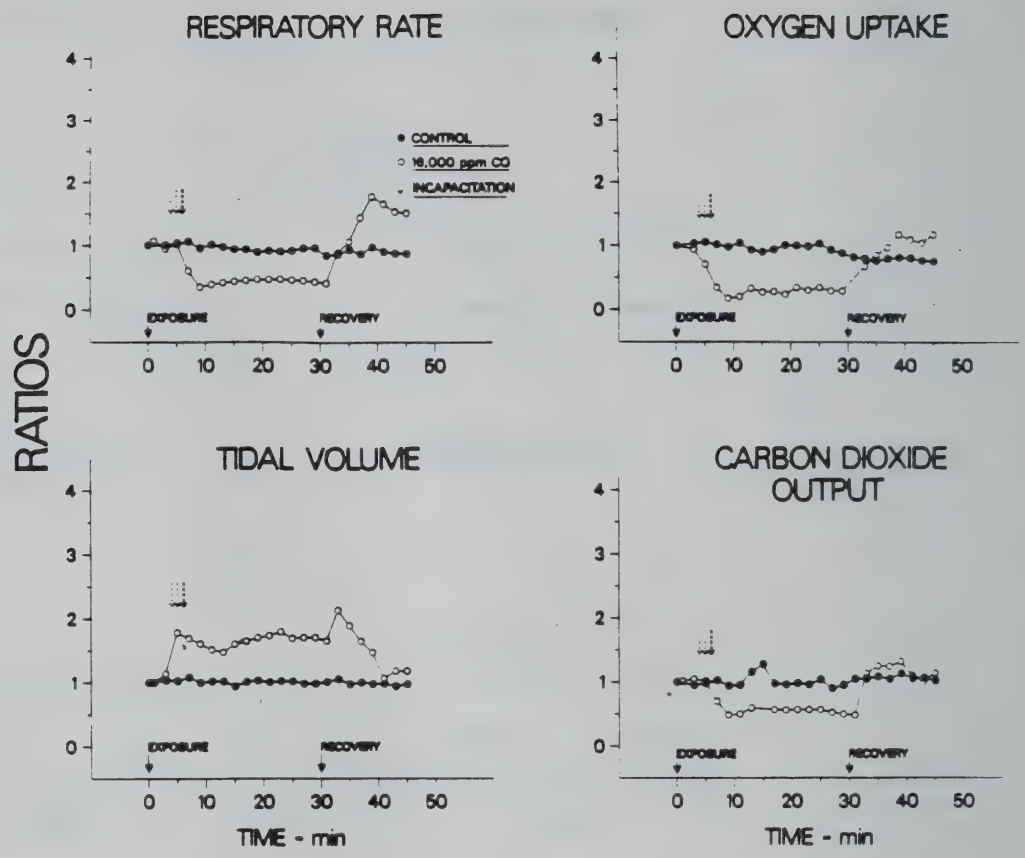


Figure 10. Physiologic measurements, f, Δ P, $\dot{V}O_2$, and $\dot{V}CO_2$ of guinea pigs during 30 minsedentary air control (n = 4) and 16,000 ppm CO (n = 4) exposures followed by 15 min air recovery. All animals were S-incapacitated and none died. The data (means) of each group was normalized by their respective average values and expressed as ratios. The % CV were less than 35% for control and less than 30% of the means for CO exposed animals for all physiologic parameters.

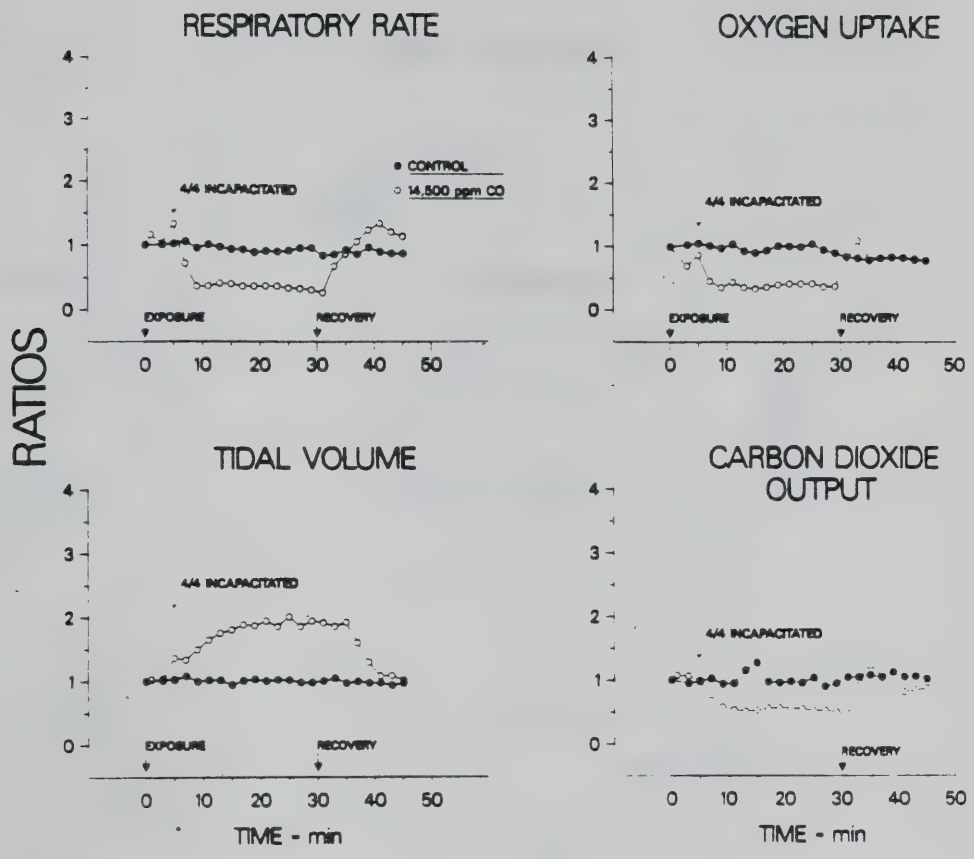


Figure 11. Physiologic measurements, f, ΔP, $\dot{V}O_2$, and $\dot{V}CO_2$ of guinea pigs during 30 min sedentary air control (n = 4) and 14,500 ppm CO (n = 4) exposures followed by 15 min air recovery. All animals were S-incapacitated and none died. The data (means) of each group were normalized by their respective average values (Table 3) and expressed as ratios. The % CV were less than 35% for control and less than 30% for CO exposed animals for all physiologic parameters.

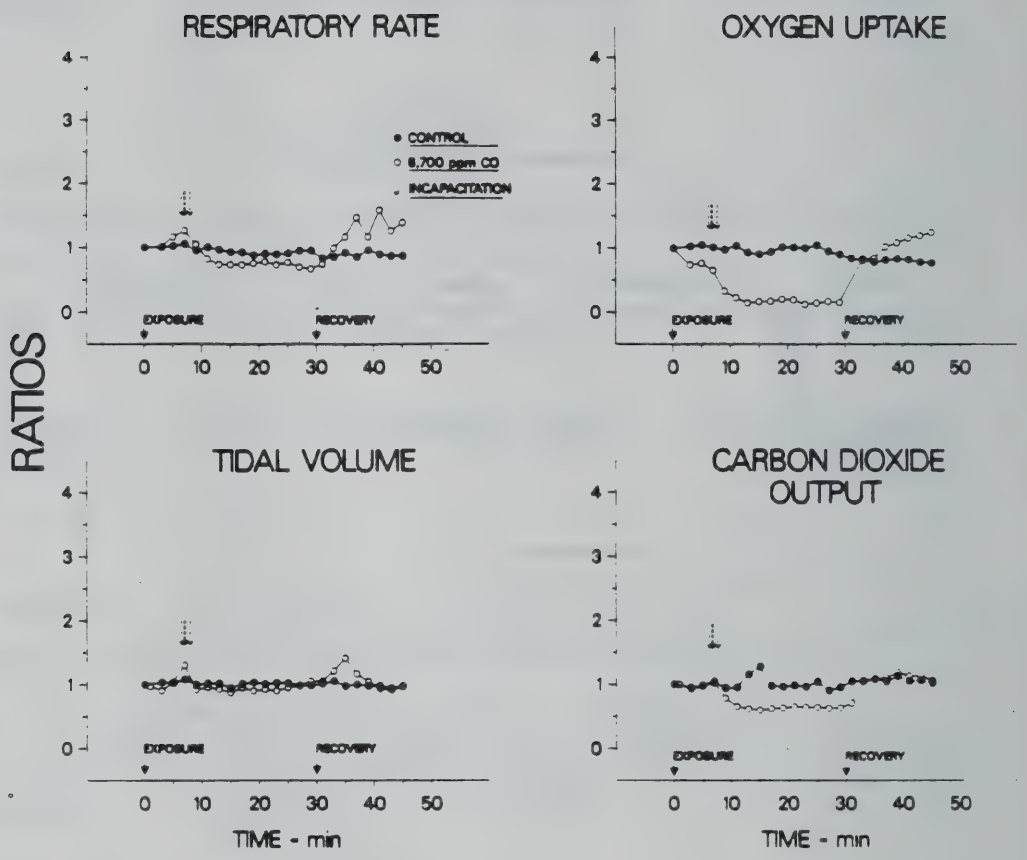


Figure 12. Physiologic measurements, f, ΔP, $\dot{V}O_2$, and $\dot{V}CO_2$ of guinea pigs during 30 min sedentary air control (n = 4) and 8,700 ppm CO (n = 4) exposures followed by 15 min air recovery. All animals were S-incapacitated and none died. The data (means) of each group were normalized by their respective average values (Table 3) and expressed as ratios. The % CV were less than 35% for control and less than 30% for CO exposed animals for all physiologic parameters.

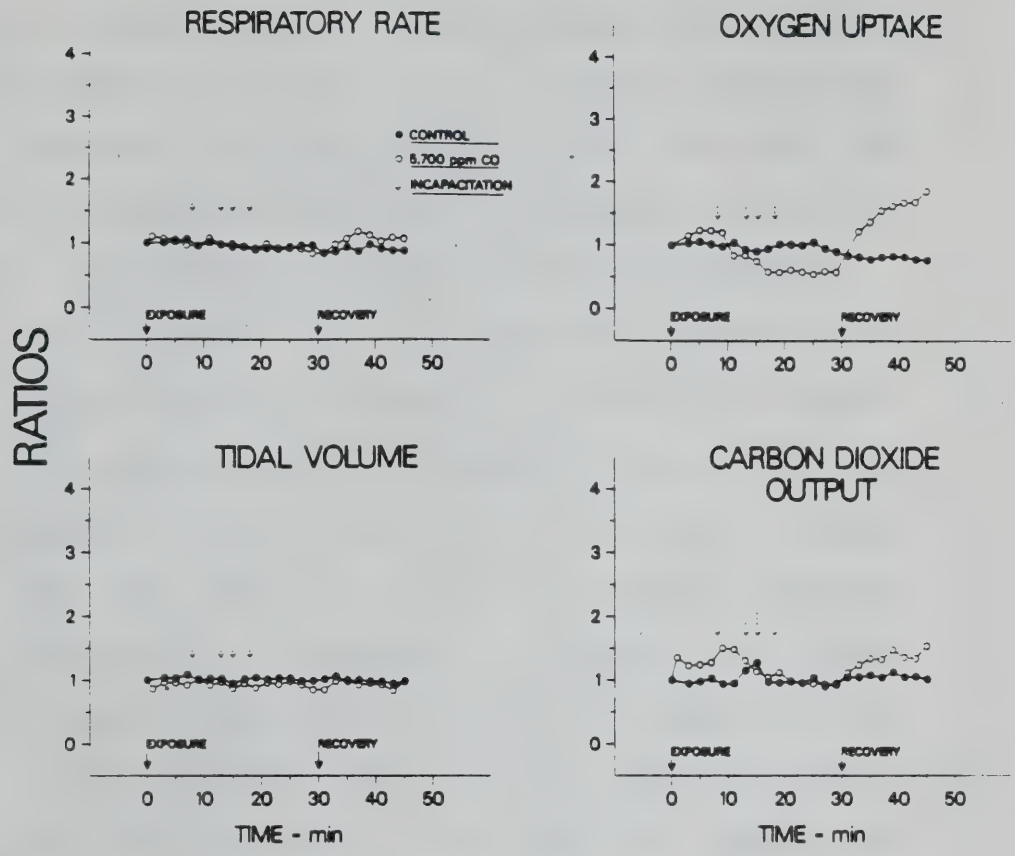


Figure 13. Physiologic measurements, f, ΔP, $\dot{V}O_2$, and $\dot{V}CO_2$ of guinea pigs during 30 min sedentary air control (n = 4) and 5,700 ppm CO (n = 4) exposures followed by 15 min air recovery. All animals were S-incapacitated and none died. The data (means) of each group were normalized by their respective average values and expressed as ratios. The % CV were less than 35% for control and less than 30% for CO exposed animals for all physiologic parameters.

the variation observed between animals. The data from each concentration of carbon monoxide exposed guinea pigs (n = 4) were also normalized using average baseline values in Table 3 and expressed as ratios similar to the air control data. The coefficients of variation were less than 30% for each physiologic parameter throughout the exposure/recovery protocol regardless of CO exposure concentration.

Figures 8 through 13 present the changes in f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ during sedentary exposures to CO and demonstrate that its effect was concentration dependent. Values for f, $\dot{V}O_2$ and $\dot{V}CO_2$ declined within the first 10 minutes of exposure and then remained at a plateau. For example, 19,000 ppm CO produced dramatic depressions in these physiologic parameters, while f and $\dot{V}CO_2$ were near normal during exposure to 5,700 ppm CO. Coincidental to a decline in f, $\dot{V}O_2$ and $\dot{V}CO_2$, the tidal volume increased as the exposure concentration to carbon monoxide increased. All of these physiologic events indicative of asphyxiation, surrounded both the occurrence of S-incapacitation and death. Regardless of exposure concentration, all exposed guinea pigs were S-incapacitated, and 100% and 75% of the animals died during 19,000 ppm and 17,500 ppm CO exposures respectively. The mean times to S-incapacitation and death for sedentary animals exposed to carbon monoxide are presented in Table 4. The time to S-incapacitation is shown to be concentration dependent (Figure 14), and the Ct values determined from these data ranged from 62,640 to 84,800 ppm.min. The LC_{50} and 95% confidence interval were calculated to be 17,129 (16,332-17,966) ppm CO based on % death listed in Table 4 as calculated by the method of Weil (1952).

Table 3

Baseline values[a] for respiratory rate, tidal volume, O_2 uptake and CO_2 output for each group of guinea pigs prior to sedentary exposures to carbon monoxide

CO ppm	f (breaths/min) X ± SD, C.V.%	ΔP (ml) X ± SD, C.V. %	O_2 (ml/kg/min) X ± SD, CV %	CO_2 (ml/kg/min) X ± SD, C.V. %
Air Control (n = 8)	107.4 ± 21.5, 19.9	.170 ± .040 21.7	30.3 ± 4.5, 14.7	26.9 ± 6.5, 23.9
19,000 (n = 4)	97.5 ± 3.1, 3.1	.163 ± .026, 16.2	21.5 ± 3.1, 14.6	21.6 ± .98, 4.5
17,500 (n = 4)	105.3 ± 17.4, 16.6	.163 ± .015, 9.2	19.1 ± 1.2, 6.3	18.9 ± 1.5, 7.8
16,000 (n = 4)	103.5 ± 7.4, 7.2	.131 ± .012, 9.2	15.1 ± 2.2, 14.6	19.4 ± 2.3, 11.8
14,500 (n - 4)	108.5 ± 15.8, 14.6	.138 ± .022, 16.1	21.5 ± 3.2, 15.0	22.6 ± 1.2, 5.1
8,700 (n = 4)	100.0 ± 10.8, 10.6	.198 ± .040, 20.8	26.4 ± 3.7, 13.9	28.5 ± 3.7, 12.9
5,700 (n = 4)	122.0 ± 21.2, 17.4	.201 ± .050, 24.9	22.1 ± .726, 3.3	24.5 ± 6.7, 27.3

[a] These values were used as "baseline" for each group to calculate the ratios presented in figures 8 through 13

TABLE 4

Percent incapacitation[a] and death, and time to effect for groups of guinea pigs exposed to carbon monoxide while sedentary and during exercise

SEDENTARY EXPOSURES					EXPOSURES DURING EXERCISE			
CO ppm	% S-Incapacitation (n = 4)	Time To S-Incapacitation (min) X ± SD, C.V.%	% Death[b] (n = 4)	Time to Death (min) X ± SD, C.V.%	CO ppm	% E-Incapacitation (n = 4)	Time To E-Incapacitation (min) X ± SD, C.V. %	% Death (n = 4)
19,000	100	3.5 ± .58, 16.5	100	11.1 ± 4.1, 37.3				
17,500	100	4.0 ± .82, 20.4	75	21.8 ± 8.5, 39.0	8,290	100	4.4 ± .25, 5.7	0
16,000	100	5.3 ± .96, 18.2	0	N[c]				
14,500	100	5.0 ± .90, 0	0	N				
8,700	100	7.2 ± .55, 7.7	0	N				
5,700[d]	100	13.6 ± 4.3, 31.7	0	N	2,200	100	11.9 ± 3.6, 30.6	0

[a] Note that the definition of incapacitation is different for sedentary vs exercise exposures, see text.

[b] LC_{50} (95% CI) = 17,129 (16,332 - 17,996), calculated by the method of Weil, 1952, based on the % death listed.

[c] The letter N indicates no event

[d] At this concentration it becomes more difficult to observe a clear incapacitation effect.

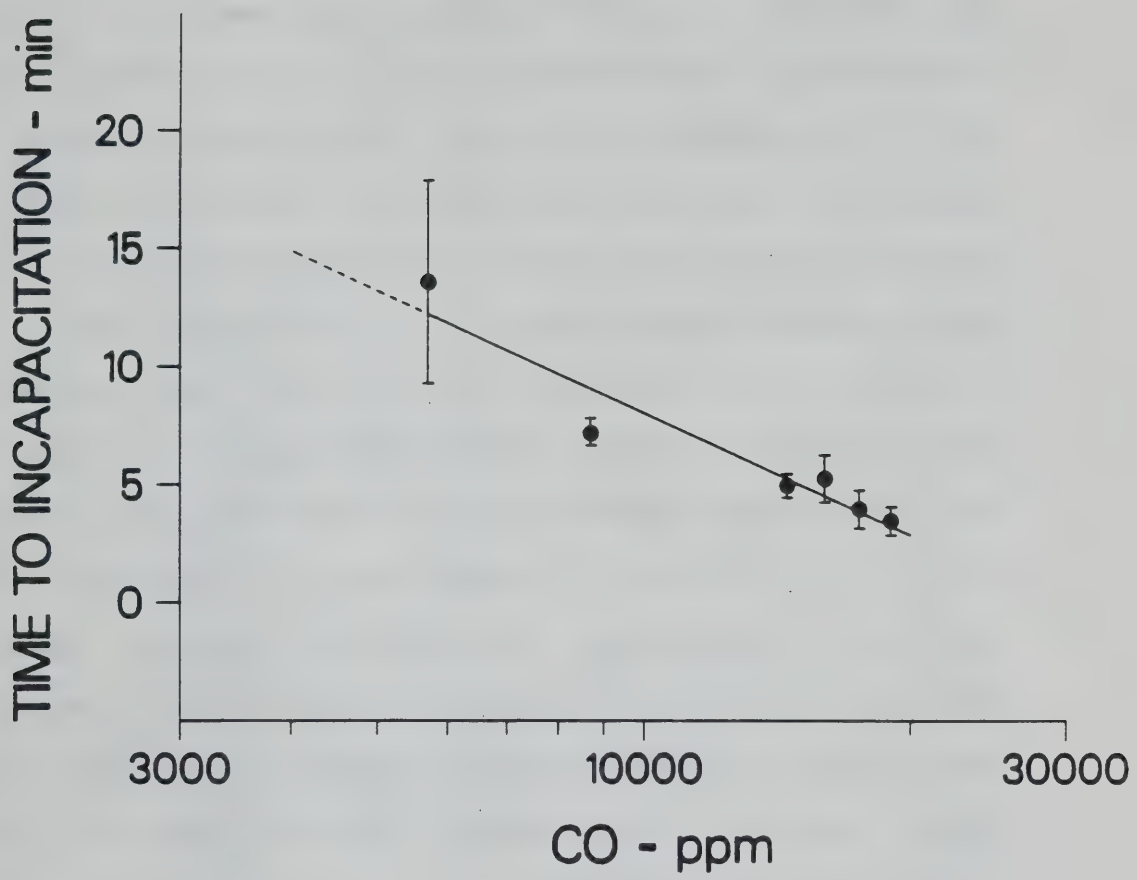


Figure 14. Concentration/response relationship (linear regression) between exposure concentration of CO (5,700 to 19,000 ppm) and mean ± SD time to S-incapacitation (min) of sedentary animals at each CO concentration (n = 4). Slope was significantly different from zero, r^2 = .92. Equation for the line was calculated to be: Y = -17.2 log X + 76.9.

2. %COHb Measurements From Normal and Cannulated Guinea Pigs

The average baseline physiologic values from those guinea pigs that were cannulated for arterial carotid blood sample (n = 16) for %COHb measurements at S-incapacitation and at death via cardiac puncture, as well as for a matched set of normal non-cannulated guinea pigs (n = 16) sampled for blood only at death, are presented on Table 5. No significant statistical difference was found between the two sets of average values (Student t-test, $p \leq .05$, Armitage, 1971). Based on this finding, the cannulated guinea pigs were not considered to be compromised due to surgery. Time to S-incapacitation, death, % death and %COHb at these times for normal and cannulated guinea pigs are shown in Table 6. The times to S-incapacitation were comparable between the two groups of guinea pigs. The cannulated guinea pigs appeared to be more sensitive to carbon monoxide reflected in an increased percent death at the 16,000 and 14,500 ppm exposure concentrations and shorter time to death at all concentrations. This may have been a consequence of blood sampling (approximately 1 ml) at S-incapacitation. The average %COHb and SD at S-incapacitation measured from the cannulated guinea pigs (n = 16) were 85.9 $\pm$ 5.8, and at death from both sets of animals (n = 12), 93.5 $\pm$ 4.6.

C. Exposures During Exercise

1. CO and Air Control Exposures

Values for f, Δ P, $\dot{V}O_2$, $\dot{V}CO_2$ measurements for air control and two exposure concentrations of carbon monoxide (8,290 and 2,200 ppm) are presented in Figures 15 through 19. Similar to the sedentary data, these data were normalized by the average baseline value for each physiologic parameter (Table 7) and expressed as ratios. By ten minutes

Table 5

Baseline values[a] for respiratory rate, tidal volume, O_2 uptake and CO_2 output for groups of normal and cannulated[b] guinea pigs prior to sedentary exposures to carbon monoxide

Guinea Pig Type	f (breaths/min) X ± SD, C.V.%	ΔP (ml) X ± SD, C.V.%	O_2 (ml/kg/min) X ± SD, C.V.%	CO_2 (ml/kg/min) X ± SD, C.V.%
Normal (n = 16)	102.9 ± 11.9, 11.6	.149 ± .023, 15.6	19.5 ± 3.3, 16.7	20.6 ± 2.2, 10.6
Catherized (n = 16)	108.9 ± 16.8, 15.5	.175 ± .038, 21.9	26.7 ± 5.2, 19.4	25.1 ± 5.4, 21.3

[a] These values were statistically compared (normal vs cannulated) to determine whether the pre-exposure physiologic state of cannulated guinea pigs were compromised by surgery, see text

[b] Guinea pigs fitted with carotid artery cannulas a minimum of 24 hours prior to exposure

TABLE 6

% COHb at S-incapacitation[a] and death and time to effect data from sedentary, normal and cannulated[b] guinea pigs exposed to carbon monoxide

CO ppm	Time to S-Incapacitation (min) X ± SD, C.V.%	% COHb at S-Incapacitation. X ± SD, C.V.%	% Death (n = 4)	Time to Death (min) X ± SD, C.V.%	% COHb at Death X ± SD, C.V.%
			NORMAL GUINEA PIGS		
19,000	3.5 ± .58, 16.5	N[c]	100	11.1 ± 4.1, 37.0	95.6 ± 3.2, 3.4
17,500	4.0 ± .82, 20.4	N	75	21.8 ± 8.5, 39.0	96.5 ± 3.1, 3.2
16,000	5.3 ± .98, 18.2	N	0	No death	N
14,500	5.0 ± 0, 0	N	0	No death	N
			CANNULATED GUINEA PIGS		
19,000	4.1 ± .07, 1.7	91.7 ± 4.4, 4.7	100	9.7 ± .57, 5.9	94.9 ± 4.2, 4.4
17,500	4.6 ± .21, 4.5	84.7 ± 2.3, 2.7	75	14.3 ± 3.6, 25.3	92.7 ± 4.5, 4.9
16,000	4.5 ± 1.6, 36.0	84.1 ± 5.9, 6.9	50	13.0 ± 4.2, 32.6	91.2 ± 4.5, 4.9
14,500	4.3 ± .54, 12.5	85.1 ± 7.5, 8.9	75	17.0 ± 9.6, 56.0	94.4 ± 6.8, 7.2

[a] See text for definition of S-incapacitation during sedentary exposures

[b] Guinea pigs fitted with carotid artery cannulas a minimum of 24 hours prior to exposure

[c] The letter N indicates no measurement

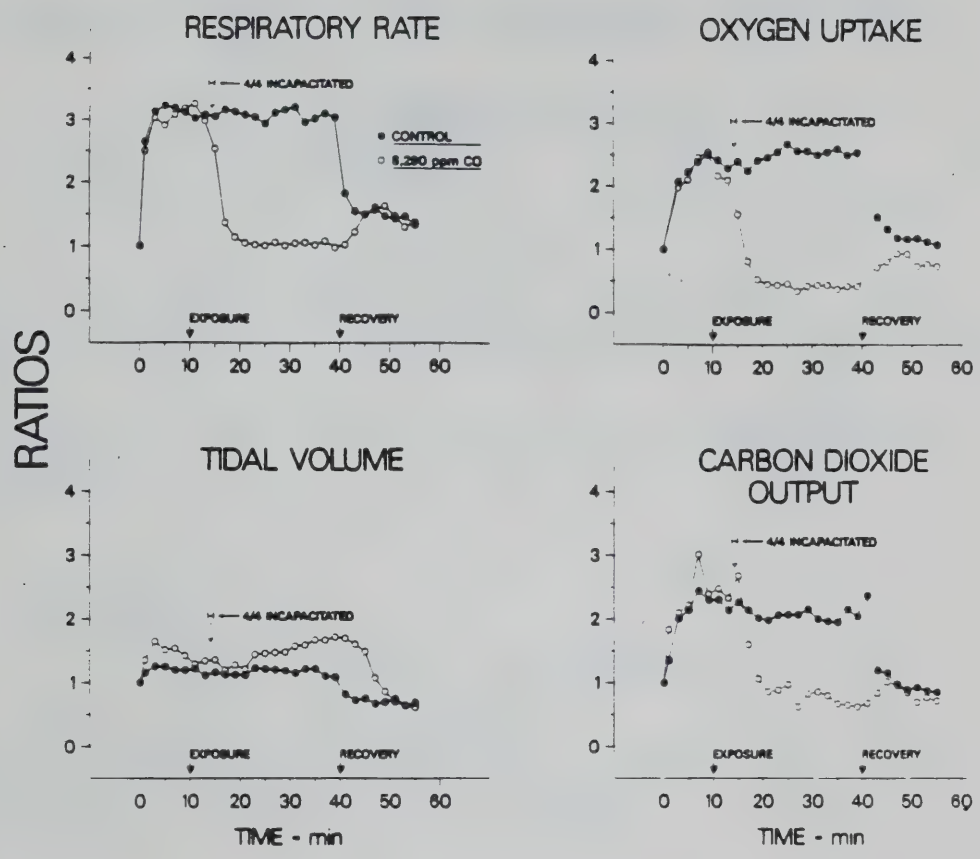


Figure 15. Physiologic measurements, f, ΔP, $\dot{V}O_2$, and $\dot{V}CO_2$ of guinea pigs during 30 min air control (n = 8) exposures and 8,290 ppm CO (n = 4) while following a "55-minute running protocol" on an ergometer (see text). The CO exposed animals became **E**-incapacitated at mean time of 4.4 min. Both air control and CO data was normalized by respective average baseline values and expressed as ratios. The % CV for control and CO data was less than 30%.

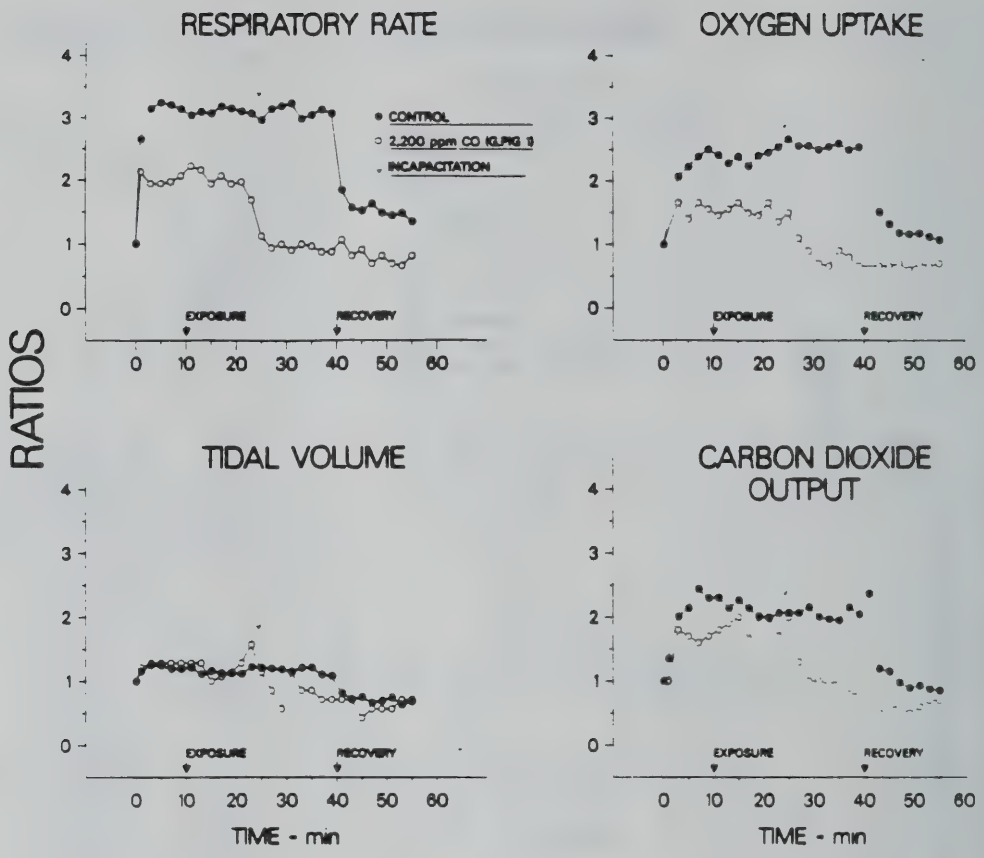


Figure 16. Physiologic measurements, f, Δ P, $\dot{V}O_2$ and $\dot{V}CO_2$ of air control (n = 8) and one guinea pig exposed to 2,200 ppm CO during a "55 minute running protocol" on an ergometer. The CO exposed guinea pig was E-incapacitated at 14.5 minutes into the 30 min exposure. Both air control and CO data was normalized by respective average baseline values and expressed as ratios (see text). The % C.V. for control data was less than 30%.

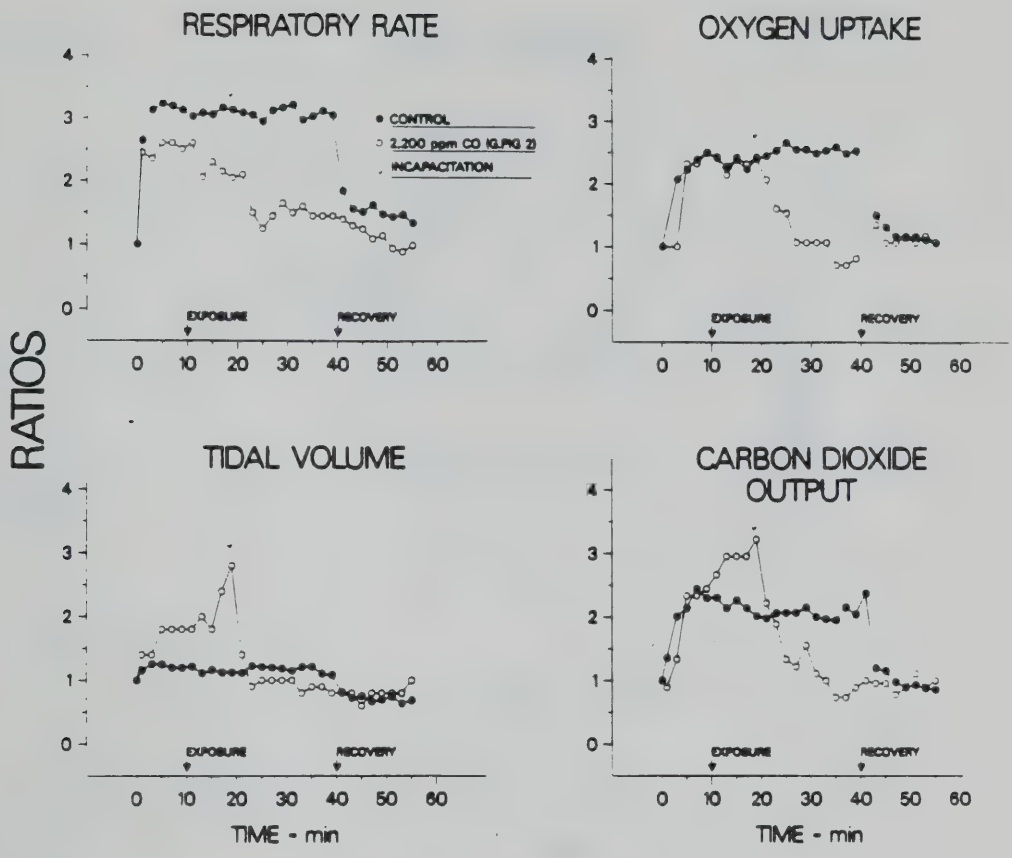


Figure 17. Physiologic measurements, f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ of air control (n = 8) and one guinea pig exposed to 2,200 ppm CO during a "55 minute running protocol" on an ergometer. The CO exposed guinea pig was **E**-incapacitated at 8.5 minutes into the 30 min exposure. Both air control and CO data was normalized by respective average baseline values and expressed as ratios (see text). The % C.V. for control data was less than 30%.

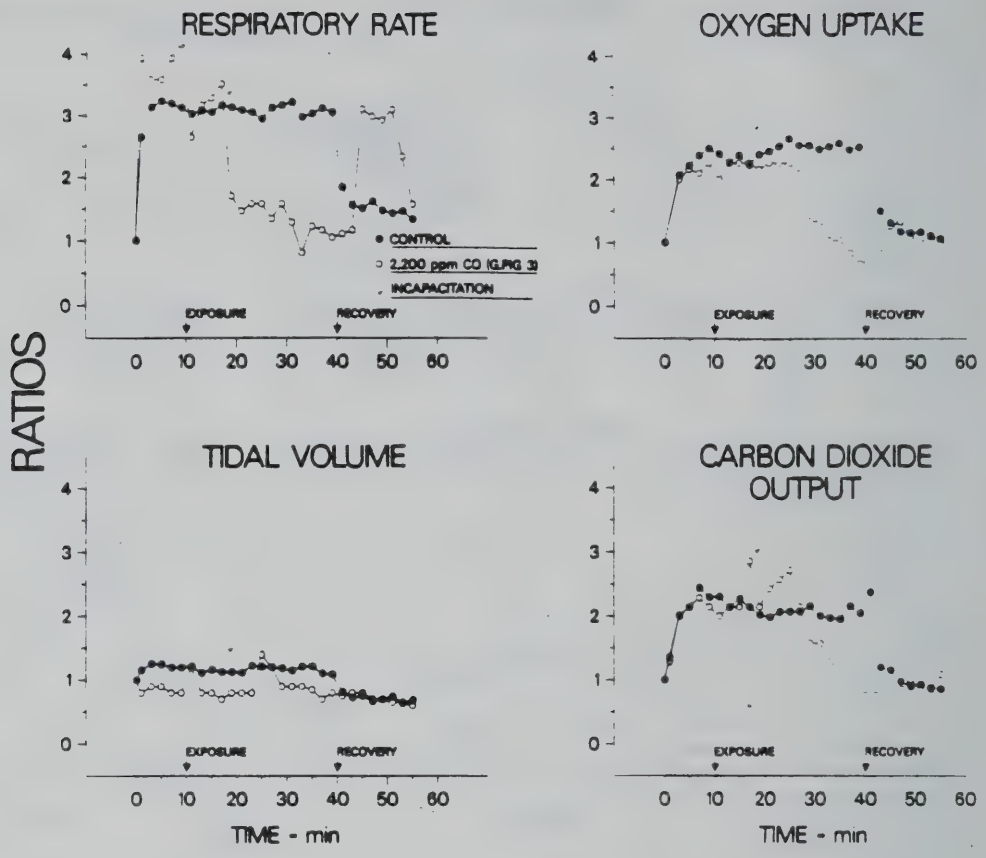


Figure 18. Physiologic measurements, f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ of air control (n = 8) and one guinea pig exposed to 2,200 ppm CO during a "55 minute running protocol" on an ergometer. The CO exposed guinea pig was **E**-incapacitated at 15.5 minutes into the 30 min exposure. Both air control and CO data was normalized by respective average baseline values and expressed as ratios (see text). The % C.V. for control data was less than 30%.

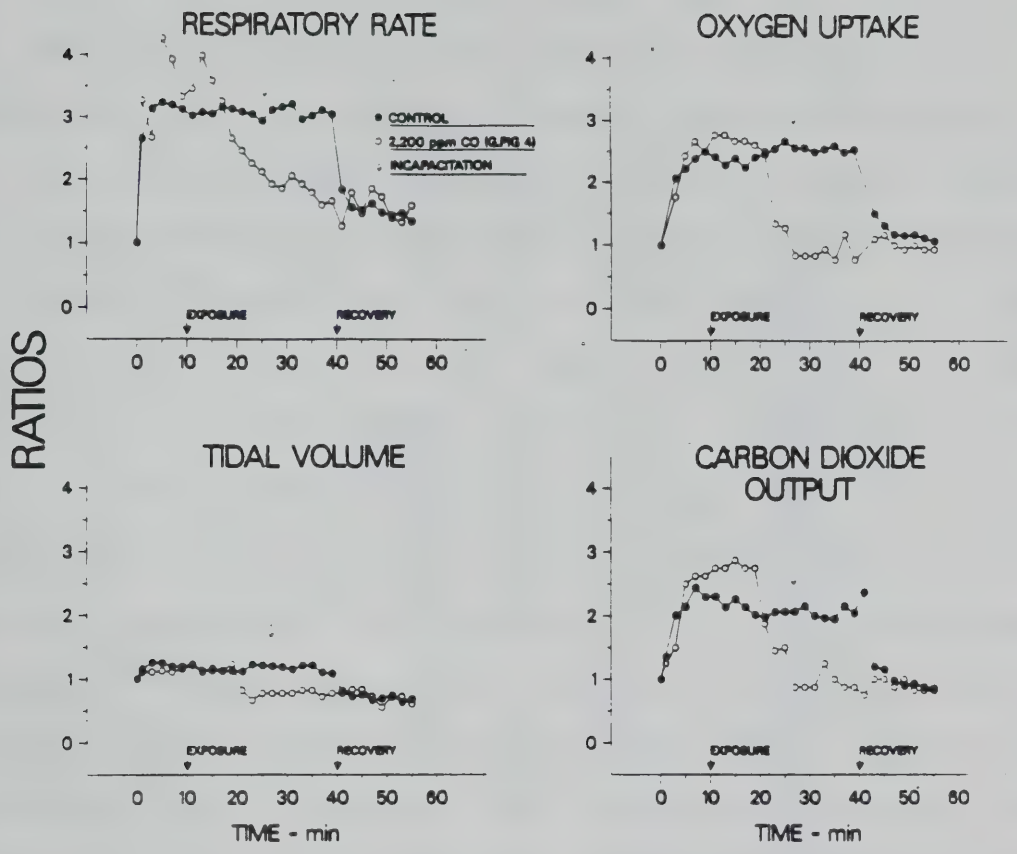


Figure 19. Physiologic measurements, f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ of air control (n = 8) and one guinea pig exposed to 2,200 ppm CO during a "55 minute running protocol" on an ergometer. The CO exposed guinea pig was **E**-incapacitated at 9.0 minutes into the 30 min exposure. Both air control and CO data was normalized by respective average baseline values and expressed as ratios (see text). The % C.V. for control data was less than 30%.

TABLE 7

Baseline values[a] for respiratory rate, tidal volume, O_2 uptake and CO_2 output for each group of guinea pigs prior to carbon monoxide exposures during exercise

CO ppm	f (breaths/min) X ± SD, C.V.%	△P (ml) X ± SD, C.V.%	O_2 (ml/kg/min) X ± SD, C.V.%	CO_2 (ml/kg/min) X ± SD, C.V.%
Air Control (n = 8)	93.1 ± 14.1, 15.2	.232 ± .050, 21.4	17.6 ± 3.2, 18.1	21.4 ± 15.2, 3.3
8,290 (n = 4)	100.0 ± 10.0, 10.0	.242 ± .031, 12.8	18.9 ± 6.4, 33.8	21.7 ± 5.1, 23.7
2,200 (n = 4)	105.0 ± 38.1, 36.3.	.273 ± .055, 20.3	19.4 ± 4.9, 25.3	22.9 ± 2.8, 12.3

[a] These values were used as "baseline" for each group to calculate the ratios presented in figures 15 through 19

into the running protocol, the air control guinea pigs (n = 8) increased their f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ by approximately 3.25, 1.25, 2.5, and 2.0 times baseline respectively. These elevations remained at a fairly constant level until the wheel was stopped at protocol minute-40. At this time, the physiologic parameters returned to pre-run baseline levels. The coefficients of variation of the mean values for each of the air control parameters were less than 25%. During the first ten minutes of the running protocol, the 4 guinea pigs that were subsequently exposed to 8,290 ppm CO followed a physiologic profile similar to that of the air control animals (Figure 15). This profile continued until protocol minute 14.4 or 4.4 minutes into the exposure to CO which was the average time to **E**-incapacitation and the time the wheel was stopped. At this time, respiratory rate declined and reached a plateau at a level comparable to baseline; both $\dot{V}O_2$ and $\dot{V}CO_2$ declined and plateaued below baseline. These levels were maintained until recovery in air for 15 min when near or slightly above baseline values were then reached. ΔP was also reduced subsequent to **E**-incapacitation. This decrease in ΔP, however, was alternatively followed by an increase as the exposure to carbon monoxide and asphyxiation progressed. Although both f and $\dot{V}O_2$ fell dramatically at **E**-incapacitation, they also showed evidence of a decline prior to 4.4 minutes. This decline, as well as the behavioral events of "sitting" and "slipping" of the guinea pig rather than its running, warned of impending E-incapacitation. All guinea pigs were **E**-incapacitated, but there was no occurrence of death. Except for a few isolated data points the coefficient of variation for each of the physiologic parameters among the 8,290 ppm CO exposed guinea pigs were less than 25%.

As a result of the differences in time to **E**-incapacitation (14.5, 8.5, 15.5, 9.0 min) and consequently when the ergometer wheel was stopped, the 4 guinea pigs exposed to 2,200 ppm CO were graphed individually (Figures 16 through 19). Except for f (which was sometimes lower), the average physiologic responses of these 4 animals were close to the control at 10 minutes into the protocol and immediately prior to exposure. Their f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ were 2.5, 1.31, 2.2 and 2.25 times baseline respectively. Their response was similar to the animals exposed to 8,290 ppm CO but with less pronounced, f, ΔP, $\dot{V}O_2$, and $\dot{V}CO_2$ declines subsequent to **E**-incapacitation. Only ΔP of guinea pig 3 (Figure 17) does not show any major changes throughout the protocol and exposure. During recovery all parameters approached control levels in all animals, and no animals died.

Average time to **E**-incapacitation and death for CO exposed guinea pigs during running as well as when sedentary are presented on Table 4. The Ct values from 2,200 and 8,290 ppm CO exposures during exercise were calculated to be 26,180 and 36,476 ppm · min respectively.

The **E**-incapacitation that occurred as a result of carbon monoxide exposured during running were different from those **S**-incapacitations that occurred while guinea pigs were sedentary. When sedentary animals became incapacitated, they fell to their sides, clearly "flat out" and did not move except for eye blinks or rapid body movements which were characteristic of a convulsive state prior to death in those animals that died. When a guinea pig collapsed from running in the ergometer, it was a dramatic event; the animal was also "flat out". However, when the wheel was slowly turned shortly after incapacitation was called, the guinea pig could not walk. Yet, a few minutes later the animal showed

signs of recovery. This was particularly easy to recognize for each of the guinea pigs exposed to 2,200 ppm CO. These animals blinked their eyes, lifted their head, looked around and occasionally took a step or two. However, when the ergometer wheel again was slowly turned the guinea pig could still not negotiate a walk nor could running be initiated.

2. HCl Exposures

Running guinea pigs exposed to 411, 530, 572, 591, or 652, ppm HCl were quickly incapacitated within 45 sec. Respiratory rate (from visual observation since no physiologic monitoring was done with HCl exposures) following E-incapacitation averaged 18 breaths/min. Guinea pigs exposed to the last 4 concentrations died within 4 minutes of exposure. A summary of observed results from HCl exposures is outlined on Table 8. Gross examination of the lungs from all HCl exposed animals showed hemorragic spots on all lobes.

3. Mean Distance Traveled During CO or HCl Exposures

The mean distance traveled $\pm$ SD for 2,200 ppm and 8,290 ppm CO exposed guinea pigs were 315 m $\pm$ 96, and 117 m $\pm$ 6.7 respectively. The five guinea pigs exposed to the range of HCl 411 to 591 ppm traveled a mean distance of 16.7 m $\pm$ 2.0.

TABLE 8

Approximate time (sec) to effect during hydrogen chloride exposures in exercising guinea pigs

Observations	HCl (ppm) 411	530	572	591	652
E-incapacitation	35	45	40	35	35
Dark eyes and skin of ears and paws	<60	<60	<60	<60	<60
Convulsions and Choking	N[b]	180	90	60	90
Death	N	240	150	140	150
Recovery [a] (walk)	420 (post-exposure)	N	N	N	N

[a] This guinea pig was exposed to HCl for 210 sec, then to room air.

[b] The letter N indicates no event.

DISCUSSION

A. Development of the Guinea Pig Ergometer Model

Despite the fact that the majority of toxic inhalation exposures of laboratory animals have occurred at rest, there is strong justification to conduct studies during exercise. Exposed human populations are usually active with above resting ventilation rates under very normal conditions, such when engaged in work or play, jogging, and certainly in extreme examples where individuals are attempting to escape from fire smoke or other toxic fumes resulting from industrial accidents. In contrast, exposed experimental animals are at rest or maintaining minimal states of activity as they are confined in their exposure chambers.

During exercise, several events occur that may influence the physiologic response to a given concentration of an airborne toxicant. An elevated $\dot{V}_E$ would result in an increased internal exposure per unit time. A resting $\dot{V}_E$ of an average human is approximately 7L/min and during acute heavy exercise over 100L/min has been reported (de Vries, 1983). In addition to an increased $\dot{V}_E$, other factors during exercise may alter toxicological response to an inhaled contaminant. These include greater depth of breath, decreased time of interface between inspired air and upper airways, increased mouth breathing (humans) and the potential to override reflex mediated breathing patterns, such as

respiratory rate depression, that is normally initiated by irritant exposures (ASTM, 1984; Brain, 1970; Yokoyama and Frank, 1972). In general, an increase in flow rate will alter the deposition of inhaled particles and reactive gases to all regions of the respiratory tract. This may consequently result in a redistribution of the dose pattern and a change in the total combined dose delivered to all regions. These factors are likely to produce an increased physiologic response to a toxicant. Studies in both humans and animals have supported this. (DeLucia and Adams, 1977; Folinsbee, et al., 1977; Sheppard, et al., 1981; Silverman, et al., 1976; Mautz, et al., 1982). Exercise can effectively increase the rate of toxicant delivery by a factor of 10.

For the purpose of examining the effect of exercise as a modifier of the toxicological response to an inhaled toxicant relative to sedentary exposures and to evaluate performance and escape potential, the guinea pig ergometer has been developed. The development of the guinea pig ergometer and exposure system was a formidable task. To establish an appreciation of the overall running inclination of the guinea pig, several test approaches were made.

The running behavior of guinea pigs was first observed inside the roughened surface of a large plexiglas tube. This consideration was based on the remarkable enthusiasm displayed by both rats and mice running in activity wheels, likened to those found in a pet shop or actually used for experimental purposes (Crane, C.R., et al., 1977; Kishitani and Nakamura, 1979). The guinea pigs displayed no interest in walking the interior of the plexiglas tube.

A reconstructed conveyor belt was examined next. The idea stemmed from various devices described in the literature that was used to

successfully condition guinea pigs to run. Guinea pigs were trained to run primarily on variable speed and angled motor driven tread-mills. Histochemical changes in muscles in response to exercise was the basis of most of these studies (Maxwell, et al., 1973; Faulkner, et al., 1972; Bernard, et al., 1971). Cellular changes in bone (Williams and Brandt, 1984) and changes in adipose tissue (Pitts, et al., 1971) were also studied in the exercising guinea pig. Pasquis, et al. (1970), examined $\dot{V}O_2$ in several species of rodents including the guinea pig. During experimentation with the conveyor belt guinea pigs were inclined to run most reliably at either end of the machine where the textured belt was advanced by two drums. This observation led to the development of an apparatus whereby the guinea pigs could run over the top of a wheel with a similar arc as the drums of the conveyor. The first model was similar to the current design diagramed in Figure 1 except that the wheel was not enclosed. A 2.2L stationary curved plexiglas chamber served to confine the running animal at the top of the wheel and functioned both as an exposure chamber and whole body plethysmograph. A flexible gasket and silicone grease between the rubber running tread and exposure box, and the application of tension by two adjustment screws sealed the exposure box to the wheel. A delicate balance of tension was required to seal the system yet permitted the wheel to turn to exercise the animal. Although this design was adequate for preliminary studies with carbon monoxide and monitoring the physiologic response of exposed guinea pigs, its long term technical integrity for body plethysmographic measurements (f and ΔP) was questionable. The seal had a tendency to change during the course of an exposure. In addition, the seam on the rubber tread hit the gasket on the exposure box two times (front and

rear of the box) per revolution of the wheel. As a result, respiratory event signals were continously obstructed by associated large pressure changes with each hit. With this design, respiratory measurements were only possible when the wheel was abruptly stopped during the course of the running protocol of the guinea pig.

In other apparatus designs where $\dot{V}O_2$ was monitored in active rodents such as while swimming (McArdle, 1966) or while running on a tread-mill (Pasquis, et al., 1970; Mautz, et al., 1985), the entire activity area was enclosed, but its volume was too large to be used as a whole body plethysmograph for measuring f and ΔP. An enclosed system of low volume is needed for whole body plesthysmography as well as for rapid mixing of introduced toxicants. This led to the design and construction of the current ergometer where a keystone shaped exposure chamber completely enclosing the running animal and wheel. This was certainly an improved design, although additional modifications (new door gasket and selectively placed bolts and wing nuts) to inhibit the leakage of gas and loss of pressure about the door and axis of the wheel were required. The guinea pig ergometer presented in Figures 1 and 2 resulted from these final modifications. This apparatus and the exposure and analytical system shown in Figure 3 provided the basis for exercise vs. sedentary exposure toxicity comparisons as well as performance evaluation in guinea pigs exposed to CO or HCl.

B. Significance of f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ Measurements

Although the measurement of a variety of physiologic parameters would contribute to an evaluation of toxicity and performance in animals exposed to inhaled toxicants, it was thought that f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$

were the most relevant. Both mice (Matijak-Shaper and Alarie, 1982, ASTM, 1984) and guinea pigs Burleigh-Flayer et al., (1985) have responded to the inhalation of asphyxiants and irritants by very specific changes in respiratory patterns. The appearence of these patterns, therefore, during an exposure would help to characterize the nature of toxic insult. An assessment of $\dot{V}_E$ would provide information relevant to dose or uptake of the toxicant. From a physiologic standpoint $\dot{V}O_2$ is an overall index of energy expenditure, influenced by an integration of many physiologic processes. If a system is insulted during a toxic exposure, metabolic exchange as measured by $\dot{V}O_2$ and $\dot{V}CO_2$ is likely to be compromised. From the standpoint of model design, measurement of $\dot{V}O_2$ and $\dot{V}CO_2$ provided a basis for standardizing the physiologic state of exposed animals from one experiment to another, and subsequently from one species to another. All of the aforementioned physiologic parameters can also be measured non-invasively.

C. Comparison of Sedentary Exposures and Exposures During Exercise

1. Air Control, Sedentary

The ventilatory variables f and ΔP determined from the sedentary guinea pigs in this study are consistent with those described in the literature (Mortola and Noworaj 1985; Wong and Alarie, 1982). Oxygen consumption measurements were similar to those determined by Ferguson (unpublished data) using a similar exposure and O_2 analytical system. Mortola and Noworaj (1985) have reported ventilatory variables and oxygen consumption in a variety of newborn and adult mammals, including the guinea pig and human (Table 9). Their oxygen data has been recalculated and expressed in ml/kg/min for the purpose of comparison

TABLE 9

VENTILATORY VARIABLES AND OXYGEN CONSUMPTION IN NEWBORNS AND ADULT MAMMALS

Species	BW	$\dot{V}_E$	V_T	F	$\dot{V}_{O2}$	$\dot{V}_{O2}^*$	References for ventilatory variables	References for $\dot{V}_{O2}$
Newborns								
Mouse	2.5	2.3	0.016	146	0.073	29.2	Mortola and Fisher (1980) Mortola (1984)	Fitzgerald (1953)
Hamster	6.4	3.0	0.038	83	0.197	30.8	present study	Bartlett and Areson (1977)
Rat	7.2	6.6	0.068	109	0.141	19.6	Mortola and Fisher (1980) Mortola (1984)	Taylor (1960)
Rabbit	79.3	55.4	0.83	76	1.67	21.1	Mortola and Fisher (1980) Mortola (1984)	Hull (1965)
Guinea Pig	91.1	76.1	0.67	123	1.73	18.9	Mortola and Fisher (1980) Mortola (1984)	Adamsons et al (1969)
Cat	118.8	95.0	1.43	80	2.53	21.3	Mortola and Fisher (1980) Mortola (1984)	Hill (1939)
Dog	297.2	242	2.59	72	3.95	13.3	Mortola and Fisher (1980) Mortola (1984)	Crighton and Pownall(1974)
Pig	1167	666	14.2	46	19.8	16.9	Mortola and Fisher (1980) Mortola (1984)	Mount and Rowell (1960)
Man	3388	863	20	43	23.4	6.9	Cross (1949) Haddad et al. (1979) Fisher et al (1982)	Talbot (1938)
Adults								
Mouse	20	25	0.15	163	0.55	27.5	Guyton (1947)	Brody (1945)
Hamster	100	49	0.97	53	1.45	14.5	Guyton (1947) Chapin (1954)	Malan and Hildwein(1965)
Rat	283	168	1.75	91	4.1	14.5	Guyton(1947) McCutcheon (1951) Lai et al. (1978) Leong et al. (1964) Bartlett and Tenny (1979)	Brody (1945)
Rabbit	2170	450	6	79	17	7.8	Maskrey and Nicol (1980)	Lee (1939)
Guinea Pig	481	158	1.8	87	6.2	12.9	Guyton (1947) McCutcheon (1951)	Brody (1945)
Cat	2700	626	24	26	30.6	11.3	Gautier (1978) Wang et al. (1948)	Brody (1945)
Dog	27300	3670	280	18	151	5.5	McCutcheon (1951) Amoroso et al. (1964)	Brody (1945)
Pig	225000	37000	-	-	527	2.3	Brody (1945)	Benedict(1938) Brody (1945)
Man	69000	7260	584	13	241	3.5	Guyton (1947) Hemingway et al. (1956)	Brody (1945)

BW, body weight (g); $\dot{V}_E$, minute ventilation ($ml \cdot min^{-1}$); V_T, tidal volume (ml); F, respiratory frequency ($breaths \cdot min^{-1}$); $\dot{V}_{O2}$, oxygen consumption ($ml \cdot min^{-1}$); $\dot{V}_{O2}^*$, oxygen consumption ($ml \cdot kg^{-1} \cdot min^{-1}$)

TABLE MODIFIED FROM MORTOLA AND NOWORAJ, 1985

among the different species of animals in different studies and with our results. The average resting $\dot{V}O_2$ measured in all normal guinea pigs in the present study (approximately 20 ml/kg/min) was higher than those reported both by Mortola and Noworaj (1985), and by Pasquis, et al. (1970), who determined a range of 10.3 to 18.9 ml/kg/min for the guinea pig at rest. The guinea pigs of the present study were 6-8 weeks old and not at rest but rather were free to move about and explore their exposure chamber. Their young age and this activity could account for the higher observed $\dot{V}O_2$. Compared to the human (Table 9) the $\dot{V}O_2$ of the sedentary guinea pig is 2.7 to 3.6 times higher both as a newborn and as an adult. It is about the same as the rat throughout life and from 1.6 to 2.1 times lower than the laboratory mouse.

With increasing muscular work, there is a parallel increase in $\dot{V}O_2$ and $\dot{V}CO_2$. The normal pulmonary response to muscular exercise is a precise integration of changes necessary to satisfy these increased demands. When a work load is gradually increased, the increase in oxygen demand is met by an increase in $\dot{V}_E$. An elevated $\dot{V}_E$ results from alterations in both f and tidal volume (ΔP). At low levels of work in humans, the increase in tidal volume is the major component. Tidal volume plateaus at approximately one half the vital capacity (½ of 4.8 L in a healthy male) with progressively higher work loads, while f continues to increase up to 50 breaths/min from a resting level of 10 breaths/min (Bove and Lowenthal, 1983). In contrast, the guinea pig immediately increased its f at the onset of exercise more so than ΔP. Within 10 minutes into the 55-minute running protocol f increased to nearly 3 times baseline and ΔP only to approximately 1.3 times baseline. It has been postulated that this difference between humans

and a quadrupedal animal such as the guinea pig may be related to the coupling of locomotion to respiratory rate. The quadrupedal gait producing more chest wall deformation with locomotion thus favoring a larger increase in f than in ΔP in guinea pigs (Bramble et al., 1983). Also in a furry animal such as the guinea pig, thermal polypnea would be favored (Dempsey et al., 1985). This response of the guinea pig to exercise is also in distinct contrast to their respiratory response to 10% CO_2 where ΔP normally increases by 3 times and f by 1.5 above baseline (Wong and Alarie, 1982). Their response to CO_2 is similar to the response in humans where tidal volume also increases to a larger extent than f (Alarie and Schaper, 1987).

Since in mammals metabolism is essentially aerobic, $\dot{V}O_2$ is an indicator of energy expenditure. Guinea pigs that ran the 55-minute running protocol clearly increased their $\dot{V}O_2$ and coincident $\dot{V}CO_2$ in response to progressively higher ergometer speeds and reached a steady submaximal $\dot{V}O_2$ 2 to 2.5 times that of baseline (40 - 50 ml/kg/min). Pasquis, et al. (1970) determined $\dot{V}O_{2\ max}$ for the guinea pig to be 6.5 times baseline. This increase in $\dot{V}O_2$ between rest and maximal exertion is called the scope of metabolic activity, or "metabolic scope" (Schmidt-Nielsen, 1984). Similar metabolic scopes (6 to 8 times resting $\dot{V}O_2$) have been determined for mice, rats and hamsters as well (Pasquis, et al., 1965, 1970). The guinea pigs in the present study running at 1.59 km/hr reached a $\dot{V}O_2$ between 25 and 30% of their $\dot{V}O_2$ max. The resting $\dot{V}O_2$ reported for the human adult male is approximately 3.5 ml/kg/min (Selkurt, 1976; Mortola and Noworaj, 1985; Bove and Lowenthal, 1983) and $\dot{V}O_{2\ max}$ range of 24 - 65 ml/kg/min (average 44 ml/kg/min) dependent on level of training (Astrand and Rodahl, 1977; Selkurt, 1976;

Bove and Lowenthal, 1983). The metabolic scope of the human consequently ranges from 10 to 18. If an untrained male were exercising like the guinea pig at 25% to 30% of a $\dot{V}O_{2\ max}$ of 44 ml/kg/min, he would be running at a $\dot{V}O_2$ range between 11 and 13.2 ml/kg/min. This $\dot{V}O_2$ range is associated with moderate exercise (deVries, 1983; Vogel and Gleser, 1972).

2. CO, Sedentary

Compared to other species of mammals including humans, the results of the present study show that the guinea pig is highly resistant to the asphyxiant carbon monoxide. No other data has been located in the literature involving guinea pigs exposed to CO. The Ct range for lethal effect of CO in sedentary exposed guinea pig (62,640 to 84,800 ppm.min) is significantly higher than those reported for other sedentary and also active animals ($\simeq$ 35,000 ppm.min, See Table 4). The LC_{50} in guinea pigs (17,129 ppm CO) is approximately 5 times the LC_{50} of 3,000 to 3,500 ppm CO for the mouse (Matijak-Shaper and Alarie, 1982; Esposito, 1987) and 3 times the LC_{50} (4,600 ppm CO) of the rat for a 30 minute exposure (Levin, et al., 1987). The $\dot{V}O_2$ and f of the mouse are each approximately twice that of the guinea pig (Table 9) and is likely to contribute to the difference in uptake of CO and in its toxic effects. The rat, however, has a similar respiratory rate and $\dot{V}O_2$ as the guinea pig both as a newborn and adult. The difference in lethality reported in these two species and higher resistance to CO by the guinea pig cannot be explained on the basis of $\dot{V}O_2$ and $\dot{V}_E$ alone. Preliminary work with low oxygen and hydrogen cyanide (unpublished data) tends to support the notion of the guinea pig being more resistant to asphyxiating conditions than mice.

Carboxyhemoglobin measurements were taken from sedentary guinea pigs exposed to CO (14,500 to 19,000 ppm) both at S-incapacitation and death (Table 6). Over the course of a 30 minute exposure S-incapacitation (100% of animals) occurred 2 to 6 times earlier than the mean time to death, and some animals did not die. The %COHb measured at death (93.5%) was higher than that associated with S-incapacitation (85.9%) although the difference is small and not statistically significant. Subsequent to S-incapacitation an observed decline in f coincident with progressive asphyxiation, is apparent and death then resulted at just a slightly higher %COHb. The %COHb from rats exposed to carbon monoxide (resulting from flaming Douglas fir Birky, et al., 1980) ranged from 40 to 80% COHb at incapacitation (foot flexion, Packham, et al., 1977) and was approximately 86% COHb at death (Levin, et al., 1987). The wide range of %COHb at incapacitation is based on some of the mechanistic difficulties associated with the foot flexion apparatus (personal experience) and often subjective determination of incapacitation. If an objective model established incapacitation in the rat, both the incapacitating and lethal %COHb might be similar to the guinea pig, again with death following incapacitation. Carboxyhemoglobin measurements were taken from CO exposed mice by Esposito and Alarie (1987). The lethal concentration (3,000 ppm) for these mice resulted in approximately 65% COHb, much lower than has been found in the guinea pigs of this study (93.5%), rats (86% and 87.4%) from the studies of Birky, et al. (1980) and Packham, et al., (1977) and human suicide victims when CO is the primary toxicant such as when running a car in a closed garage (95%) Esposito (1987). It has been commonly stated that humans are severely compromised, at a %COHb range of 30-40%

(Kaplan, et al., 1984; Kimmerle, 1974) and probably more so at 50% (Haldane 1895). Also 50% COHb is often taken as a lethal level in humans. The average COHb level in fire deaths occuring in Allegheny County during the past 3 years was found to be 63% (Esposito, 1987) but the range is wide and complicated by the presence of HCN and probably also by oxygen deficient at atmospheres. The average %COHb in fire deaths was reported at 45.8% by Anderson et al. (1981), again with a wide range and the presence of HCN. The reason for the difference in the lethal %COHb among the mouse, rat, guinea pig and human is of interest. In preliminary studies, samples of whole blood were each exposed to 100% CO in vitro for a given time. The %COHb was consistently highest in the guinea pig, followed by the human, and then the mouse. The guinea pig/human and mouse/human %COHb ratios were approximately 1.07 and .61, respectively (Malek and Esposito, unpublished data). Although these studies are not conclusive and certainly need to be pursued, these data suggests that the kinetics of %COHb formation in these species is different (faster in the guinea pig) and the resistance to CO exhibited by the guinea pig versus the mouse may not be explained solely on the basis of $\dot{V}_E$ and $\dot{V}O_2$. Thus, in sedentary conditions, comparing the effect of CO in rats, mice, guinea pigs and humans is complicated by the fact that death occurs at different COHb levels and these lethal COHb levels are produced by different exposure concentrations of CO. This can be seen in Table 10 which summarizes the findings just discussed. From this table the lethal CO concentration for guinea pigs is twice the concentration required for humans while the guinea pig lethal COHb level is 14% higher than humans.

Table 10

Differences in lethal levels of COHb in different species and the CO exposure concentration necessary to produce these levels

	Mice	Rats	Guinea Pigs	Humans
LC_{50}[a] (ppm)	3,000[b]	4,500[c]	17,129[d]	5,000 or 8000[e]
%COHb at LC_{50}	64[b]	84[c]	94[d]	50 or 80[f]

a Exposure duration of approximately 30 min with deaths occuring during exposure or shortly after

b From Esposito (1987)

c From Levin, et al. (1987)

d Present study

e Calculated values (from Stewart, 1973) to produce either 50% or 80% COHb respectively within 30 minutes in a sedentary adult

f 80% COHb was taken when CO was the only or major constituent (Esposito, 1987; Haldane and Priestley, 1935) while 50% COHb was taken when CO was a major constituent among other toxicants that were likely to contribute smoke, i.e., from a fire. For comparison with the other species where pure CO is used, 80% COHb should be used for humans.

Unlike mice exposed to CO concentrations around their LC_{50} (Matijak-Schaper, 1982), guinea pigs similarly exposed about their LC_{50} were fast to recover following exposure. During recovery the $\dot{V}O_2$, $\dot{V}CO_2$ and f of the guinea pig increased rapidly to levels between one and two times above baseline. Thus the guinea pig was not only resistant to high concentrations of CO during exposure, but also had the physiologic integrity to compensate for the insult post exposure. This is another factor to take into consideration when trying to explain survival of victims rescued from fires.

3. CO during exercise

Based on air control data from guinea pigs that followed the 55-minute running protocol resulting in a 2 to 3 fold increase in f, $\dot{V}O_2$ and $\dot{V}CO_2$ it was assumed that exercising guinea pigs would be at least twice as sensitive to CO as sedentary exposed animals. This was the justification for examining 2,200 ppm and 8,290 ppm CO during running exposures as these concentrations were approximately 2 to 3 fold less than 5,700 and 17,500 ppm CO concentrations previously studied in sedentary guinea pigs. Time to effect (incapacitation and death) data were compared for these exposures in Table 4. During the 17,500 ppm CO sedentary exposure (Figure 9) guinea pigs were **S**-incapacitated at a mean time of 4.4 minutes; f, $\dot{V}O_2$ and $\dot{V}CO_2$ dramatically declined and ΔP alternatively increased. These physiological events are indicative of severe asphyxiation (Matijak-Schaper and Alarie 1980) that surrounded the death of 3 out of 4 animals. As expected, the 8,290 ppm CO exposed running animals, similar to the 17,500 ppm sedentary exposed guinea pigs, were **E**-incapacitated at approximately 4 minutes. These animals, however, showed significantly less signs of asphyxiation (after their **E**-

incapacitation and stopping the running wheel) and no death occurred. Despite the same time to incapacitation in these two aforementioned experiments, the nature of incapacitations of each were different. Sedentary animals exposed to CO simply sat and were not required to perform work. At the time of falling to their sides at S-incapacitation, they were clearly unconscious, and if the chamber were shaken the guinea pig barely stirred. In running exposures, guinea pigs had a high metabolic exchange. They also collapsed unconscious, the wheel was stopped and the guinea pigs continued to be exposed to CO but now in a sedentary mode at a lower metabolic level.

During the performance of muscular exercise such as running, cardiovascular changes such as increased cardiac output, preferential diversion of blood to working muscles and extraction of greater quantities of oxygen from arterial blood, take place. Under normal human resting conditions the amount of blood flowing through the skeletal muscles is approximately 15% of total cardiac output (where cardiac output is 4-6 L/min) with an O_2 extraction, or arterial venous difference ($a\text{-}\bar{v}O_2$) of 40 - 50 ml O_2/L blood. During normal maximum exercise the $a\text{-}\bar{v}O_2$ difference could be as high as 3.3 times resting or 165 ml O_2/L blood (Astrand and Rodahl, 1977). This higher utilization of the oxygen transported by the blood is attained in two major ways: (1) the flow of blood is redistributed during exercise so that the skeletal muscles, with their enhanced ability to extract O_2 may now receive 80 to 85% of the cardiac output compared to 15% at rest (2) the oxygen dissociation curve is shifted to the right so that more oxyhemoglobin is reduced (less % O_2Hb) than normally at a given PO_2. During inhalation exposures to carbon monoxide resulting in the

formation of COHb, the oxygen dissociation curve shifts to the left (less PO_2 is required to saturate available Hb). Carbon monoxide not only reduces the amount of hemoglobin available for oxygen transport, but also inhibits the unloading of oxygen in the tissues (West, 1979). Some evidence does exist, however, that compared to hypobaric hypoxia, CO hypoxia produces a lower mixed venous O_2 tension, where more O_2 was extracted (Vogel and Gleser 1972a; Klausen et al., (1968) This may be a compensatory mechanism for the increased affinity of O_2 for hemoglobin (left shift of O_2 dissociation curve) in the presence of CO (Vogel and Gleser, 1972a). Several studies have shown that $\dot{V}O_{2\ max}$ of humans exposed to low O_2 environments, is reduced approximately proportional to the resultant decrease in arterial O_2 content (Hughes, et al., 1968; Stenberg, et al., 1966; Vogel, et al., 1969). Vogel and Gleser (1972b) have reported that human subjects exposed to low (225 ppm) concentrations of carbon monoxide, (18 - 20% COHb) also have a decreased $\dot{V}O_{2\ max}$ proportional to loss in arterial O_2 content. They also report that submaximal $\dot{V}O_2$ for a given work load was the same either exposed to air or CO (gross efficiency was not affected by CO).

The guinea pigs in the present study were exercising at submaximal $\dot{V}O_2$. Regardless of increasing %COHb with time they were forced to run at the same given work load (running speed = 1.59 km/h) with the same O_2 requirements as when exercising in air. These requirements were met and maintained ($\dot{V}O_2$ and $\dot{V}CO_2$ plateau, Figures 15 to 19) until the oxygen demand was greater than the availability of O_2 resulting in failure to run (slipping and sliding) with a coincident decline in $\dot{V}O_2$ followed by collapse (E-incapacitation). Although %COHb was not measured it must have been less than 86% found at S-incapacitation particularly for the

group exposed at 2,200 ppm. Now, after E-incapacitation and therefore in a sedentary mode where the metabolic demand of skeletal muscles was lower the running guinea pigs that had collapsed were observed to recover (eyes blinking, looking about, occassional chewing and taking a step or two) never falling to their sides like the sedentary exposed animals, despite their continued exposure to CO. These observations were more common particularly in the 2,200 ppm exposed group which is not an incapacitating concentration for a sedentary guinea. Although these guinea pigs were clearly not unconscious they could not be motivated to run again and would certainly collapse if forced to exert themselves.

There have been anecdotal accounts of humans exposed to fire smoke where the victims have reported similar nonfunctional states, as were observed in the guinea pig, yet were somewhat aware of their surroundings and had the intention to escape but just couldn't and had to be rescued. Also, fire victims on numerous occasions have apparently moved some distances in the escape process but have been found dead within a few feet of an exit door. It seems that in the case of both the human and the guinea pig a critical loading of CO is required before compensatory mechanisms fail, that lead to sudden collapse and impossibility to move. The experiments by Haldane (1895) on himself show this phenomenon. At 40 - 50% COHb he could sit or stand but it was impossible for him to move or he would collapse.

4. HCl

Hydrogen chloride was selected as the prototype irritant for performance evaluation because it is a very common and dangerous constituent of fire smoke, particularly when polyvinylchloride (PVC) is

thermally decomposed (Wooley, 1971; Paciorek, et al., 1974). PVC material is currently ubiquitous and consequently has a high probability of being involved should a fire develop.

Sedentary guinea pigs were not exposed to hydrogen chloride in the present study. The most comprehensive study of this nature was carried out by Burleigh-Flayer, et al. (1985), the results of which will be compared to guinea pigs exposed to HCl while running. Burleigh-Flayer, et al. (1985) exposed sedentary guinea pigs for 30 min to HCl ranging from 320 to 1380 ppm while their ventilation was continuously monitored by a body plethysmograph.

A respiratory pattern, characterized both by a decrease in f and an extended expiratory phase due to stimulation of the trigeminal nerve and indicative of sensory irritation (Alarie, 1973) was observed within 6 min of exposure at all concentrations. Pulmonary irritation, characterized by an initial increase in f, followed by a decrease due to a pause after each expiration was observed in all animals within 20 minutes of exposure. This respiratory pattern is associated with pulmonary irritation and the stimulation of irritant receptors within the lung (Alarie, 1981). Guinea pigs exposed to 1040 ppm HCl showed signs of pulmonary irritation after 8.5 min of exposure. These results in sedentary guinea pigs exposed to HCl reported by Burleigh-Flayer, et al., (1985) were consistent with those described by investigators involving other laboratory animals (Darmer, et al., 1974); Machle, et al., 1942). Based on the results of these investigators, the LC_{50} for a 30 min HCl exposure and 16 day post exposure observation for sedentary guinea pigs is between 1380 and 4416 ppm. Flury and Zernik (1931) exposed a variety of animals, such as the dog, cat, pigeon, frog,

rabbit, rat, mouse and guinea pig to HCl. They found all species resistant to HCl except for the rabbit and guinea pig. In rabbits they report an intoxicating syndrome as "sleep inducing". The guinea pig reacted differently, as laryngeal spasm and bronchospasm seem to be the dominant effect at low concentrations of HCl. These results support an LC_{50} at the low end of the aforementioned range suggested. The LC_{50} for the mouse and rat for a 30 minute exposure and 8 day post-exposure observation period was determined to be in the range 2264 to 3086 and 4129-5352 ppm HCl respectively (Darmer, et al., 1974). The studies of Anderson and Alarie, (1980), however, reported a significantly higher lethal concentration range (10,157 ppm) for mice exposed to HCl for 30 min. However, in mice fitted with tracheal cannula to prevent the scrubbing action of the nasal mucosa the LC_{50} was reduced to 1,095 ppm (Anderson and Alarie, 1980). Consistent with the observations of Flury and Zernik (1931), with regard to acute lethality the guinea pig is considerably more sensitive than either the rat or the mouse.

The HCl-exposed guinea pigs in the present study were previously trained by the 55-minute running protocol but were not monitored for physiologic events during the exercise exposure. Based on the physiologic profile of the air control running guinea pigs it was assumed that the 5 guinea pigs exposed to HCl were at a comparable physiologic level $\dot{V}O_2$ 2 to 2.5 times baseline, when the exposure was initiated at 10 minutes into the running protocol. Although a 30 minute exposure to HCl was intended, the dramatic incapacitating and lethal effects resulted in exposures of less than 4 minutes. The observations recorded from these animals are summarized on Table 8. The **E**-incapacitation in these guinea pigs exposed to HCl was significantly

different than the E-incapacitation obtained with CO. With HCl they were coughing, gasping for air and death occurred via suffocation for guinea pigs exposed above 530 ppm. Normally the eyes of the guinea pig, similar to the albino rat and mouse, are bright red reflecting oxygenated blood. During the exposure to HCl the eyes became blantantly dark, as did the ears and paws which are normally pink. It is clear that the guinea pigs became asphyxiated apparently due to spasm of the larynx and probably the bronchi. The guinea pig that survived the 411 ppm, 3.5 minute exposure, was able to walk again after 7 minutes of recovery in air; however, it showed evidence of noisy, labored breathing. Upon gross pathological examination, the lungs of this animal, sacrificed approximately 3 hours after exposure, showed small hemorragic spots on all lobes, while hemorragic areas were more pronounced on the lungs of those guinea pigs that died during the exposure. The ppm difference between the guinea pig that lived and those that died was less than 200 ppm. Clearly a steep concentration response curve would result with further examination of HCl by this method.

Compared to sedentary exposures, the observed toxicity of HCl was significantly greater in guinea pigs exposed while running. The average concentration of HCl in this study that killed during exercise was 586 ppm. This concentration is close to half the concentration of HCl (1040 ppm) examined by Burleigh-Flayer, et al.(1985) in sedentary animals. Yet all running guinea pigs were E-incapacitated and died within 1 and 5 minutes respectively while in sedentary animals no incapacitation or death occurred during the 30 min of exposure to 1040 ppm of HCl. Death occurred only post-exposure in sedentary animals at this concentration.

In order to produce death only post-exposure, for a 30 minute exposure during exercise HCl may need to be reduced by a factor of 8 to 10 (73 to 58 ppm) from the concentration range used in this study. The increased $\dot{V}_E$ resulting from the metabolic demands of exercise may overide the protective reflex mediated respiratory rate depression normally observed in response to an irritant such as HCl. The sedentary guinea pigs exposed to HCl by Burleigh-Flayer, et al. (1985) showed this reflex reaction clearly. In addition an increase $\dot{V}_E$ would deposit a higher amount of this irritant into the respiratory tract. These factors would account for the enhanced toxicity of HCl during exercise relative to a sedentary exposure.

Based on the examination of guinea pigs exposed to HCl and the limited data for HCl exposure in man provided by Matt (1899), Flury and Zernik (1931) and Henderson and Haggard (1943) proposed that a 1000 - 2000 ppm range of HCl would be dangerous in a short period of time since the same mechanisms (laryngeal constriction and swelling and bronchoconstriction) were operating in both humans and guinea pigs. Since the human does not have the same capability to scrub this gas by the nose compared to the guinea pig, humans have the potential to absorb more at the laryngeal and bronchial levels given the same airborne concentration. The proposal of Flury and Zernik (1931) and Henderson and Haggard (1943) seem to be reasonable in that they were adopted by the National Academy of Sciences (1976). Should an exercising human be exposed to HCl the situation may also be significantly more dangerous. The following conclusions were made by Matt (1889) after studying the effects of HCl on a few active humans, which is very limited information but the only experimental data available for HCl in humans.

HCl (ppm)	Conclusions by Matt (1889)
10	Work possible without impairment
10-50	Work possible but with hinderance
50-100	Work impossible

This would also be consistent with the prediction that 300 ppm HCl would produce intolerable sensory irritation in humans (Barrow et al.1977). However, again this prediction was made from an animal model rather than from direct observation in humans.

D. Comparison of Toxic Potency of CO vs. HCl

Compared to carbon monoxide, hydrogen chloride was clearly the more potent agent under both sedentary and exercise conditions. During sedentary conditions deaths were not observed until 17,500 ppm of CO. At this concentration, 75% of the guinea pigs died during exposure, with the first animal succumbing at approximately 13 minutes. Under sedentary conditions and exposure to HCl (Burleigh-Flayer, et al., 1985), deaths were first observed above 1040 ppm HCl. HCl produced lethality in the guinea pig at a concentration approximately 13 times lower than CO produced under similar exposure conditions. The greater toxicity of HCl compared to CO appears to be more pronounced in the guinea pig during exercise. In the present study, death occurred within 5 minutes at an average concentration of 586 ppm HCl. Based on the enhanced toxicity to CO observed during exercise compared to sedentary

conditions, the concentration of CO during exercise that might produce similar effects (fast incapacitation and lethality), as the HCl experiments would likely be in the range of 10,000 to 12,000 ppm CO. If this were a correct estimate, then exposure to HCl during exercise would be 18-20 times more toxic. Thus, exercise further increased the relative toxicity of HCl versus CO if compared to the sedentary condition (13 vs 18-20 times). HCl has a direct, quick-acting irritant effect on the respiratory tract resulting in laryngeal spasm and bronchospasm which results in suffocation (asphyxiation). Exercise served to increase penetration of HCl faster and deeper in the lung and further exaggerated this irritant effect. In comparison, systemic asphyxiation by CO took longer than asphyxiation by HCl despite faster loading of CO by exercise.

In the mouse track model (Malek, et al., 1987) mice were fitted with tracheal cannulas prior to HCl exposures while running. By this surgical procedure, HCl was not scrubbed by the mouse nose and was deposited more directly into the lung. Running mice were exposed to an HCl range of 900-2150 ppm. The number of animals that were incapacited was concentration dependent and 3 died at the highest concentration. Regardless of exposure concentration (900-2150 ppm) those mice that became incapacitated did so at 4 minutes. Running mice were also exposed to CO (2,500 to 7,200 ppm) and incapacitation was also concentration dependent. Mice were incapacitated at a mean time of 4 minutes at 6,000 ppm but no deaths occurred. Given the same exposure conditions of this model, it is predicted that much higher concentrations of CO would be necessary to produce death. From these data again, exercise appears to exaggerate the toxicity of HCl more than for CO.

E. Comparison of Sublethal Responses to Other Escape Models

The Introduction section of this thesis reviewed the behavioral models that have been developed with the intention of evaluating the potential to escape from fire smoke environments. The designs of these models feature sublethal endpoints that have little to do with the escape process. They fail to examine the capability of a test animal to move a required distance to escape. The elaborate baboon escape/avoidance model has methods that would test the decision making ability of these animals upon exposure to CO or HCl. If possible, the "ideal" model might involve a psychological/cognitive assessment paradigm, as this undoubtedly does affect overall behavior, but to be included only in addition to a definitive test of physiological integrity. Despite severely debilitating exposures to hydrogen chloride, for example, the baboons were able to differentiate among auditory and visual cues, pull appropriate levers, take a few steps to clean air and by operational definition, "escape". The escape component was too simplistic. These animals would never be physiologically capable to move great distances (work) if challenged to do so.

Due to the inquisitive nature of mice, they conveniently ran on their own volition in the mouse track model (Malek et al., 1987). This design led to the recognition of early (< 2 min) toxic effects where distance traveled/time decreased in a concentration dependent fashion. In addition to this sublethal response, incapacitation was observed. Since the guinea pig needs to be enticed to run, and the speed at which they ran was fixed, a graded decrement in their running speed like that determined in the mouse was not possible. However, distance traveled before incapacitation could be determined, as the travel speed 1.59 km/h

was known. An "all or nothing" incapacitation response associated with many behvaioral models has been critized in support of a graded response shown in the mouse track model (Malek, et al., 1987). However, collapse of a guinea pig subsequent to running a quantifiable distance in a given toxic environment is equally relevant to escape evaluation. Incapacitation-collapse in the guinea pig ergometer model is an irrefutable objective behavioral endpoint. A rapid decline in $\dot{V}O_2$ immediately prior to collapse validates the occurrence of incapacitation.

F. Extrapolation of Findings in Running Animals to Humans

Several human studies have shown that $\dot{V}O_{2\ max}$ decreases proportionately with an increase in %COHb resulting from exposure to carbon monoxide (Pirnay, et al. , 1971; Vogel and Gleser, 1972, Vogel, et al., 1972, Vogel, et al. , 1972; Horvath, et al. , 1975, Ekblom and Hvot, 1972). Elblom and Hvot (1972) not only examined changes in $VO_{2\ max}$ but also maximal work time (MWT) in CO exposed humans. In this study these parameters were examined in two groups of subjects maintained at approximately 7% and 20% COHb respectively. Compared to control exposure, the MWT observed in subjects on a bicycle ergometer decreased from 4 min 11 sec to 3 min 14 sec (24% decrease) at 7% COHb and to 2 min 27 sec (40% decrease) at 20% COHb. Similar exposure groups exercising on a treadmill decreased MWT from 5 min 32 sec in air to 4 min 4 sec (26% decrease) at 7% COHb and to 2 min 54 sec (48% decrease) at 20% COHb. Both the decreases, in MWT and $\dot{V}O_{2\ max}$ as a function of blood COHb are presented in Figure 20. Nearly a 50% reduction in work time (although running speed was not reported) occurred at 20% COHb. Although the guinea pigs in the present study were only running at 30%

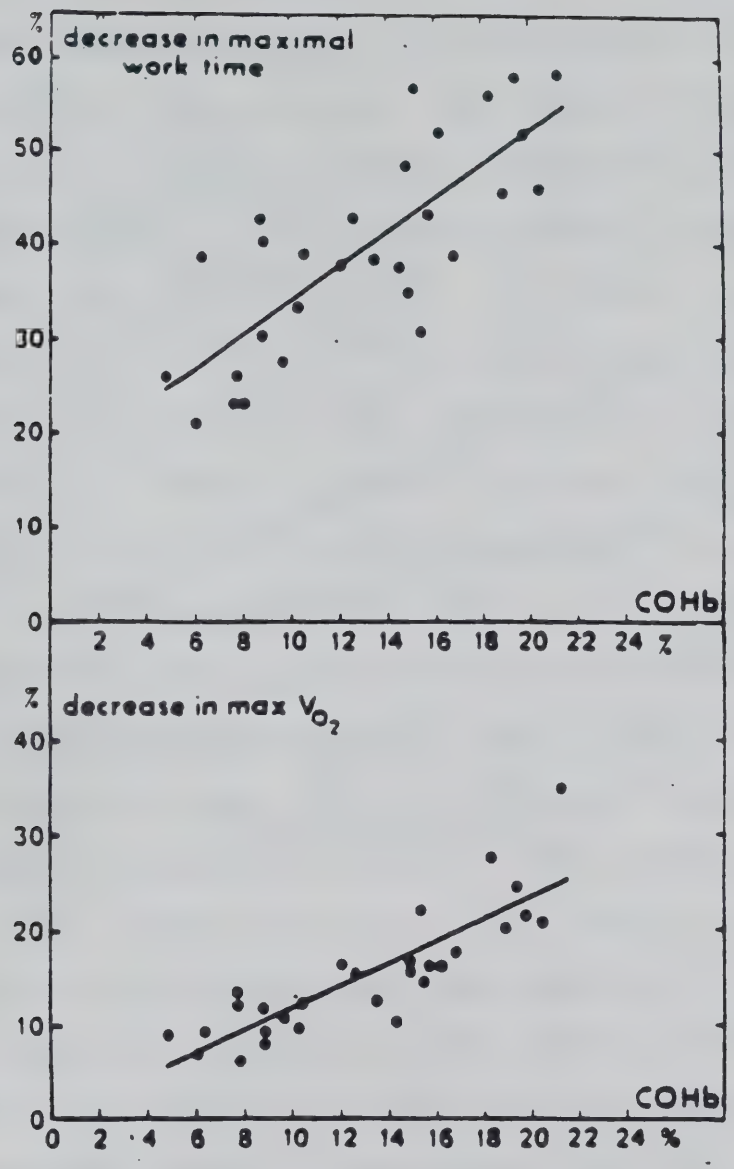


Figure 20. Relation between different levels of COHb in blood and corresponding per cent decrease in work time on a fixed maximal rate of work on the treadmill and per cent decrease in maximal oxygen uptake. From Elblom and Hvot, (1972).

$\dot{V}O_{2\ max}$, their collapse time was similar to guinea pigs exposed in a sedentary mode, yet their exposure was less than half the sedentary exposure concentration of CO (5,700 vs 2,200 ppm CO). Decreased distance traveled (work time) was also observed during a running exposure to 8,290 ppm compared to running at 2,200 ppm CO and certainly to the air exposed guinea pig able to complete the 55-minute running protocol (Table 4). Although the %COHb at incapacitation was not measured, the values would likely be the same among the CO exposed animals (2,200 or 8,290 ppm), only the time to reach this level of saturation would be different (longer at lower concentrations). Although $\dot{V}O_{2\ max}$ was not measured in the mice in the mouse track model (Malek, et al. , 1987) they were estimated to be exercising at approximately 25 to 30% their $\dot{V}O_{2\ max}$ from their running speed (Schmidt-Nielsen, 1984). Their distance traveled (work time) was also dependent on exposure concentration to CO. One major advantage that the guinea pig ergometer model has over the mouse track model is an ability to control through $\dot{V}O_2$ the level of exercise during exposure which is similar to the human experiments described by Ekblom and Hvot (1972).

Because larger animals take fewer steps, they use less energy (less O_2) to move one unit of body mass over one unit of distance. Taylor, et al. (1970) have demonstrated a linear relationship between oxygen consumption (ml O_2 kg^{-1} hr^{-1}) and running speed (km hr^{-1}) for several species of animals (Figure 21). Since the cost of running, expressed in ml O_2 kg^{-1} km^{-1} (slope) for each animal is independent of speed at which it runs, speed as a variable can be eliminated in the calculation of cost. Consequently cost of running can be directly related to body mass. The work of Taylor (1970) and more recently the work of Schmidt-

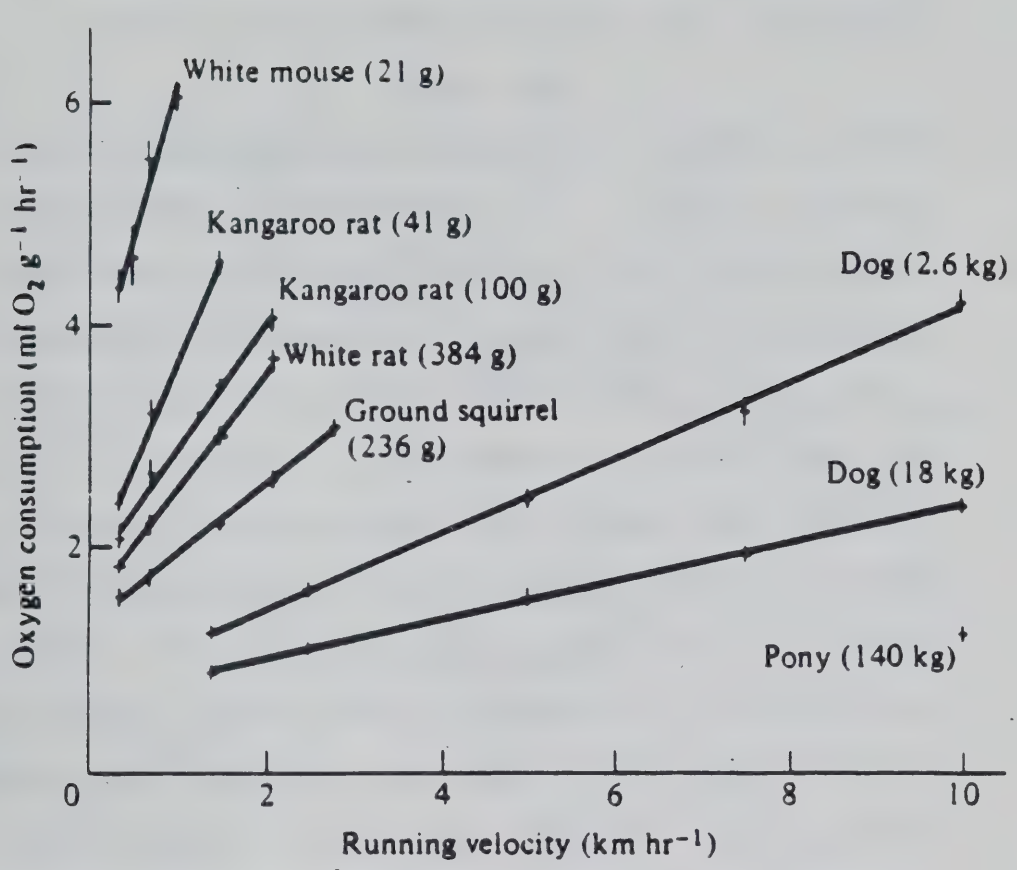


Figure 21. Oxygen consumption for running mammals increases with the speed of running. For each species, the increase is linear, but the oxygen consumption increases more steeply for a small animal than for a large animal. From Taylor et al. (1970).

Nielsen (1984) have shown an inverse linear relationship between cost and body mass among various birds, reptiles and mammals including humans (Figure 22). Cost can be calculated from the equation

$$\text{Cost} = a\ M_b^{\,c},$$

where M_b body mass in kilograms, **c** is the exponent (slope) and **a** is the intercept at unity. If 0.32 were used as the exponent reported by Schmidt-Nielsen (1984) and 8.4 as the intercept taken from Taylor (1970) the cost of a 0.017 kg mouse is approximately 14 times and a guinea pig 5 times that of a 70 kg human traveling the same distance. The larger the animal the more efficient it is. From these data it is reasonable to predict that humans in air would progress 5 meters for each meter traveled by the guinea pig. In the present study the distance traveled related to work time of the guinea pig was limited by exposure concentration to CO compared to air control. Based on the work of Ekblom and Hvot (1972) human work time would also decrease like the guinea pig, yet the human would travel 5 times further. The 5 fold difference in distance traveled before incapacitation, however, is based on the assumption that both the human and guinea pig are equally as sensitive to carbon monoxide, and they clearly are not as shown in Table 10. From this table, it is reasonable to estimate that the human would need only to be exposed to half the running guinea pig CO exposure concentration yet progress (based on the calculation of cost, ml O_2 $kg^{-1}m^{-1}$) 5 times the distance of the guinea pig. The running guinea pigs in the present study were exercising at a $\dot{V}O_2$, approximately 2.5 times resting. A human with a similar increase in $\dot{V}O_2$ would be traveling at approximately 100 m/min. From this traveling speed, time to incapacitation or total work time can be estimated as shown in Table 11.

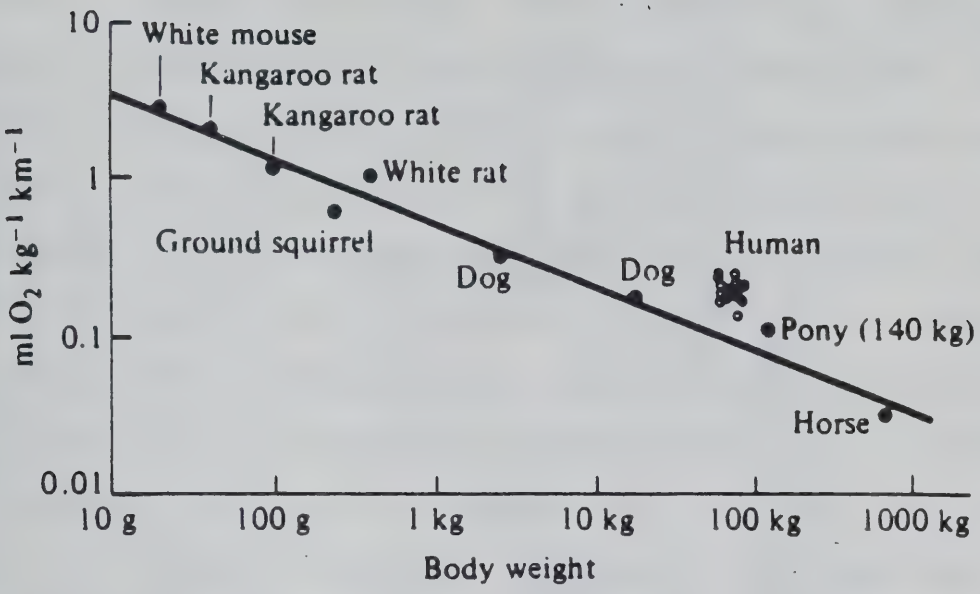


Figure 22. The cost of running, expressed as the oxygen needed to transport 1 kg of body weight over 1 km, decreases with increasing body size. Data for man (bipedal running) fall above the line representing data for mammals running on all four legs. From Taylor et al. (1970).

Table 11

Extrapolating results to humans for incapacitation due to CO during exercise in guinea pigs and comparison with predicted values in humans

Results in guinea pigs			Extrapolation to humans			Predictions of Bernard and Duker (1981) for humans[a]		
CO (ppm)	Distance traveled before incapacitation (m)	Time before incapacitation (min)	CO (ppm)	Distance traveled before incapacitation (m)	Time before incapacitation (min)	CO (ppm)	Distance traveled before incapacitation (m)	Time before incapacitation (min)
0 (air control)	795[e]	--	0	3975[b]	--	0	--	--
2,200	315	11.9	1,100[c]	1575[b]	15.8[d]	1,100[c]	1200	12
8,290	117	4.4	4,145[c]	585[b]	5.9[d]	4,145[c]	500	4

[a] Using the Coburn equation to calculate CO loading and an initial $\dot{V}O_2$ of 2 L/min corresponding to an initial traveling speed of 170 m/min. The distance and time values were approximated from Figure 2 of Bernard and Duker (1981) for 1,100 and 4,145 ppm.

[b] Estimate based on calculation of cost (ml O_2 kg^{-1} km^{-1}) in that the human would travel 5 times the distance traveled by the guinea pigs.

[c] The exposure concentrations for the guinea pig are divided by 2 since the concentration to produce the same level of COHb in humans is 0.5 that required for the guinea pig (Table 10).

[d] Assuming a traveling speed of 100 m/min corresponding to a $\dot{V}O_2$ 2 to 2.5 times resting, similar to the running guinea pig.

[e] Distance traveled for 30 min at 1.59 km/h (26.5 m/min) as given in Figure 5 for the running protocol resulting in an increase $\dot{V}O_2$ of 2.5 above resting.

Bernard and Duker (1981) modified the Coburn-Forster-Kane model, initially used to examine the role of endogenous sources of CO, and developed a theorectical model that examined the effects of carbon monoxide on humans during work. Their subjects were theoretically exposed to various inspired concentrations of CO with an initial $\dot{V}O_2$ of 2 L/min, corresponding to an initial traveling speed of approximately 170 m/min. The starting $\dot{V}O_2$ in this human model represented a work level higher than the running guinea pigs in the present study, however through the course of the CO exposure the $\dot{V}O_2$ would decline to represent an average metabolic level increase comparable to the running guinea pig. Based on this assumption, distance traveled (m) and time to incapacitation (min) were approximated for 1,100 and 4,145 ppm CO (Figure 23). Time to incapacitation was read from the graph where the distance/time profiles just began to curve to the right. Prediction of human response by the model of Bernard and Duker (1981) are also presented in Table 11.

It can be seen from this table that the extrapolation to humans made from the results obtained in guinea pigs is in good agreement with the model proposed by Bernard and Duker (1981). Scientists involved in modeling toxic hazard in fire situations could use the results of Bernard and Duker (1981) for appropriate estimates of human performance during exposure to CO.

Extrapolating the results of HCl exposure obtained in guinea pigs to humans is more difficult due to the fact that so few experiments have been done with this gas in humans. However, it would be prudent to consider 500 ppm HCl as rapidly incapacitating in exercising humans until further data to the contrary.

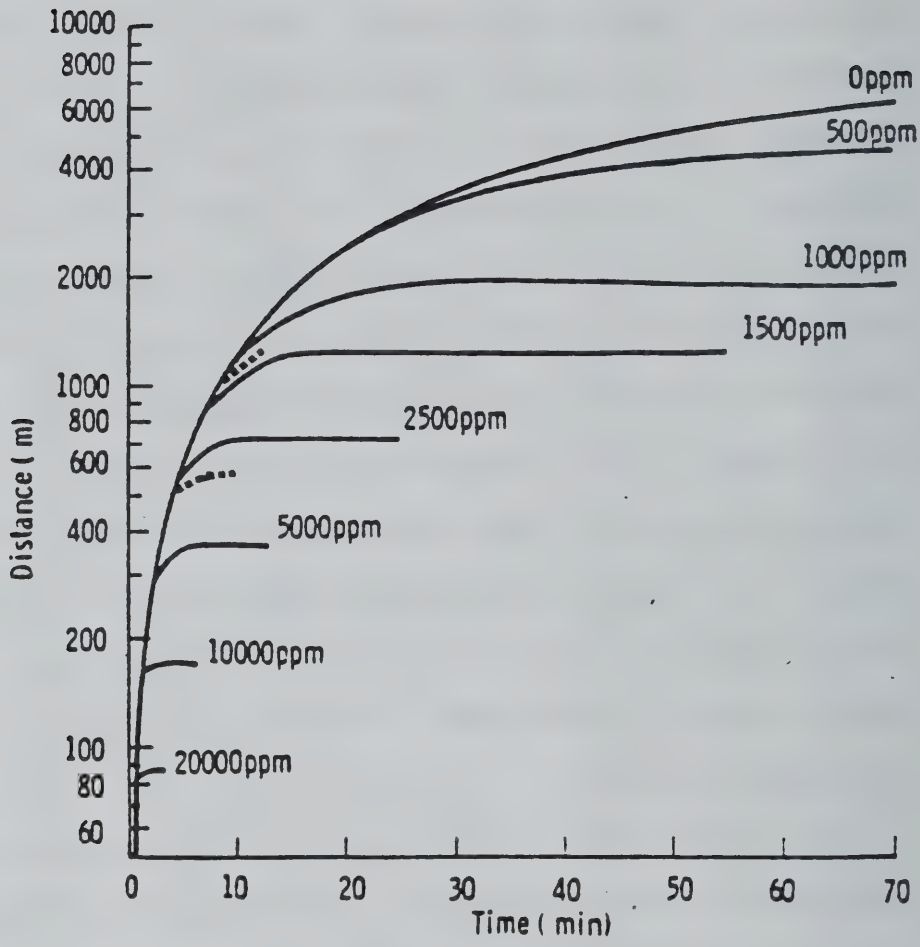


Figure 23. Calculated distance traveled as a function of time at different levels of inspired CO (ppm) with an initial oxygen consumption of 2.0 L/min. From Bernard and Duker (1981). The broken lines were added to represent 1,100 and 4,145 ppm given in Table 11.

G. Suggestions for Future Studies

Although the guinea pig has demonstrated a resistance to CO correction factors can be determined and used to relate the guinea pig to humans. The sublethal endpoint (collapse) was very sensitive to CO in that it occurred during exercise at a concentration much lower than the lethal level in sedentary animals. Some additional work with CO is suggested so that a concentration dependent distance traveled curve could be developed. A complete evaluation of other asphyxiating conditions such as hydrogen cyanide and low oxygen is also recommended.

Based on preliminary results with HCl exposures during exercise it appears that the guinea pig is an excellent animal model for irritant evaluation. The study of HCl should be pursued at exposure concentrations below 100 ppm so that both E-incapacitation and lethality would be delayed into a 30 minute exposure. It would be interesting to know if the guinea pig is debilitated at concentrations of HCl described by Matt (1889) that makes work by humans impossible.

In addition to the aforementioned pure gases, an evaluation of gas combinations and ultimately fire smoke from burning materials is of importance. An analysis of toxic hazard in fires requires these data.

The protocol described for exposures during exercise can be modified so that the evaluation of inhaled toxicants could occur at various metabolic levels as measured by $\dot{V}O_2$. Such studies would require actual technical modifications of the guinea pig ergometer. These changes would include the installation of alternative gears to the motor to increase the maximum speed from 1.59 km/h, or placing the wedge shaped running area of the exposure chamber on an angle. Both of these adjustments would serve to increase $\dot{V}O_2$ upon exercise.

CONCLUSIONS

1. Due to the need to generate fire smoke inhalation toxicity data in active animals and determine sublethal responses that would be escape predictive, two animal models have been developed: (1) the mouse track model, and (2) the guinea pig ergometer model.

2. Performance evaluation in the mouse track model for CO and low O_2 and HCl was based on two sublethal responses: (1) distance traveled/time, and, (2) incapacitation. The most important feature this model added to the existing set of behavioral models was its ability to detect an early graded deterioration in performance prior to incapacitation and death. HCl was the most potent toxicant tested.

3. The guinea pig ergometer model has provided information on both a behavioral and physiologic basis.

   a. The model measured distance traveled before incapacitation (collapse), which is directly related to the escape process.

   b. In addition this is the only model which permitted the continuous measurement of f, ΔP, $\dot{V}O_2$ and $\dot{V}CO_2$ while a guinea

pig was running during an inhalation exposure. The $\dot{V}O_2$ parameter has functioned to select a level of exercise and validate the occurrence of incapacitation.

c. From the evaluation of effects of carbon monoxide and hydrogen chloride it is clear that the toxicity of a given exposure condition increased with exercise.

d. Compared to the guinea pig, the human is twice as sensitive to carbon monoxide. Humans appear to be equally sensitive to the incapacitating effects of hydrogen chloride although the data in humans is very limited.

e. Results from the extrapolation of the exercising guinea pig data to the human is similar to currently available theoretical models that predict human response to CO. From this model, the distance traveled by a human would be five times the distance traveled by guinea pigs at a similar level of toxicity for CO.

f. By the escape predictive responses of distance traveled and incapacitation the guinea pig ergometer model can be used to evaluate pure gases, mixtures and products of combustion. This type of data is critical to an assessment of fire smoke toxicity hazard.

Appendix A

Analytic Procedures For CO and HCl

A. Carbon Monoxide

Calibration

A miran infared analyzer (Model 1A) was used to monitor carbon monoxide exposure concentration. Carbon monoxide in the range of 500 to 10,000 ppm has been successfully analyzed by the following Miran settings.

Wavelength	- 4.7 nm
Pathlength	- 14.25 metres (setting #9)
Gain	- x10
Absorbance	- 1
Time Constant	- .25

A chart recorder used to record the Miran output was set at 1 volt for full scale. During calibration the Miran was a closed 5,600 ml system, the contents circulated by a pump. A calibration curve was developed by 20 (28 ml) sequential syringe injections of carbon monoxide into the system through a septum from a 9.84% CO balance nitrogen tank. Each injection was equivalent to approximately 500 ppm CO and produced a corresponding division deflection on the chart recorder. As the number of injections in the closed Miran increased, the CO ppm increased as did the chart defection. The concentration of each injection was calculated by this equation.

$$\frac{\text{volume of syringe injection (ml)}}{\text{Miran volume (ml)}} \times \frac{\text{CO calibration tank (ppm)}}{} = \text{Syringe injection CO (ppm)}$$

$$\frac{28 \text{ ml}}{5600 \text{ ml}} \times \frac{98{,}640 \text{ ppm CO}}{} = 493.2 \text{ ppm CO}$$

A typical calibration for carbon monoxide up to 10,000 ppm may be observed in Figure A-1.

For the analysis of carbon monoxide concentration above 10,000 ppm, the chart recorded full scale was reduced to 0.5 volts and commonly 40 sequential injections of 500 ppm each produced a calibration curve up to 20,000 ppm CO.

B. Hydrogen Chloride

1. Basic Principle

Hydrogen chloride exposure concentrations were determined colometrically using a modification of the Osterreiche Stickstoffwerke method of analysis (Leithe, 1971). The Ferric thiocynate ion ($FeSCN^{2+}$) is one of the last species formed in the series of reactions that occur in the analytical procedure. These reactions are outlined in Figure A-2. The concentration dependent intensity of orange/red color due to $FeSCN^{2+}$ is measured spectrophotometrically at 460 nm and is proportional to chloride ion concentration (hydrogen chloride).

2. Reagents Required for Analysis

1. 6N HNO_3 (94.5 ml concentrated acid/250 ml H_2O)
2. $FeNH_4(SO_4)_2$ (20.0 g $FeNH4(SO_4)_2$/250 ml 6N HNO_3)
3. Hg (SCN) (2.5 g Hg(SCN)/250 ml Methanol)
4. .1N NaOH (4.0 g NaOH/1000 ml H_2O

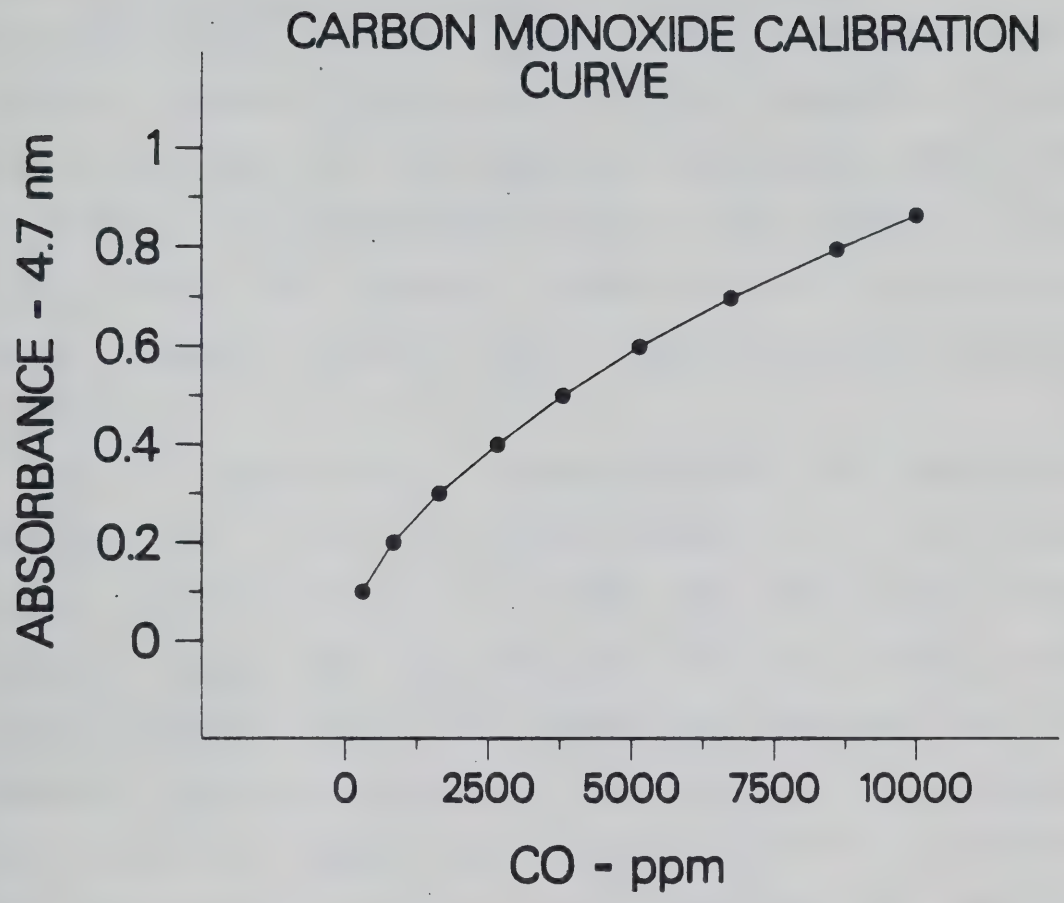


Figure A-1 Calibration curve for CO measured by Miran infared analyzer.

5. .01N NaOH (50 ml .1N NaOH/500 ml H_2O)
6. 0.1 NaCl (0.5844 g NaCl/100 ml H_2O)
7. 0.01 NaCl (10 ml .1N NaCl/100 ml H_2O)
8. 2N HNO_3 (6.3 ml concentrated acid/100 ml H_2O)

3. Calibration Curve

The standard curve for hydrogen chloride was produced by analyzing a series of solutions containing known concentrations of chloride (1 ml 0.01 N NaCl = 354.5 ug Cl^-/ml). Each of these standards were prepared in a 50 ml volumetric flask by the addition of reagents outlined on Figure A-2 and Table A-1. Each standard was read against a buffer blank at 460 nm on a spectrophotometer (Baush & Lomb Spectronic 70) and plotted against their respective concentrations of chloride (ug/ml sample). A typical calibration curve for hydrogen chloride is presented in Figure A-2.

Exposure concentrations of hydrogen chloride were determined by sampling at .95 L/min for 3 minutes through two in series midget impingers containing 30 ml of .01 N NaOH. Each impinger was analyzed separately. 5 ml were taken from the total 15 ml volume of the impinger, placed in a 50 ml flask, prepared, and absorbence read as the standards. The concentration of Cl^- was determined from the standard curve (Figure A-3). Assume a sample absorbence at 460 nm was .255, and this corresponded to 11.5 ug Cl^-/ml on the curve, HCl (ppm) was calculated as follows:

$$\text{ug } Cl^-\text{/ml from curve} \times \frac{50 \text{ ml}}{\text{sampling time (min)} \times \text{sampling rate (L/min)}} \times \frac{\text{Impinger volume (ml)}}{\text{sample from impinger (ml)}} = \text{ug } Cl^-$$

$$11.5 \text{ ug } Cl^- \times \frac{50 \text{ ml}}{3 \text{ (min)} \times .95 \text{ L/min}} \times \frac{15 \text{ ml}}{5 \text{ ml}} = 605.3 \text{ ug } Cl^-$$

$$\text{ug } Cl^- \times \frac{1 \text{ ppm}}{1.47 \text{ ug } Cl^-} = \text{ppm } Cl^- \text{ or ppm HCl}$$

TABLE A-1

Standard chloride solutions and corresponding absorbance values for a typical hydrogen chloride calibration curve

Standard Chloride	.01 N NaCl (ml)[a]	.01N NaOH (ml)	2 N HNO_3 (Drops)	$Hg(SCN)_2$ (ml)	$FeNH_4(SO_4)_2$ (ml)	ug Cl^-/ 50 ml[b] solution	ug Cl^-/ ml	Absorbance 460 nm
1	0.50	30	3	2	2	177.3	3.55	.100
2	1.00	30	3	2	2	354.5	7.09	.155
3	1.50	30	3	2	2	531.8	10.64	.208
4	2.00	30	3	2	2	709.0	14.18	.257
5	2.50	30	3	2	2	886.3	17.73	.292
6	3.00	30	3	2	2	1063.5	21.27	.328
7	4.00	30	3	2	2	1418.0	28.36	.385

[a] 1 ml of .01N NaCl = 354.5 ug Cl^-/ml

[b] After solutions are added in order to a 50 ml volumetric flask the volume is bought to 50 ml by the addition of distilled water

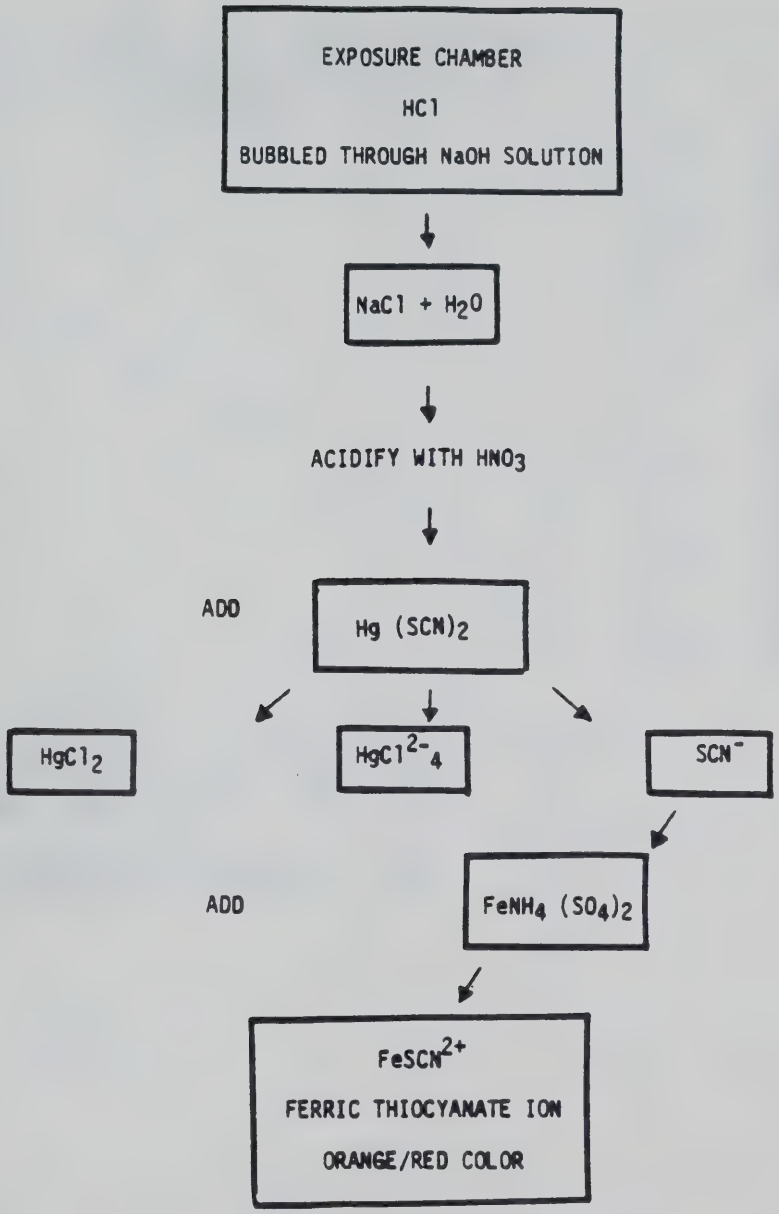


Figure A-2 Sequence of reagent additions and reactions involved with the analysis of HCl by the Osterreiche Stickstoffwerke method (Leithe, 1971).

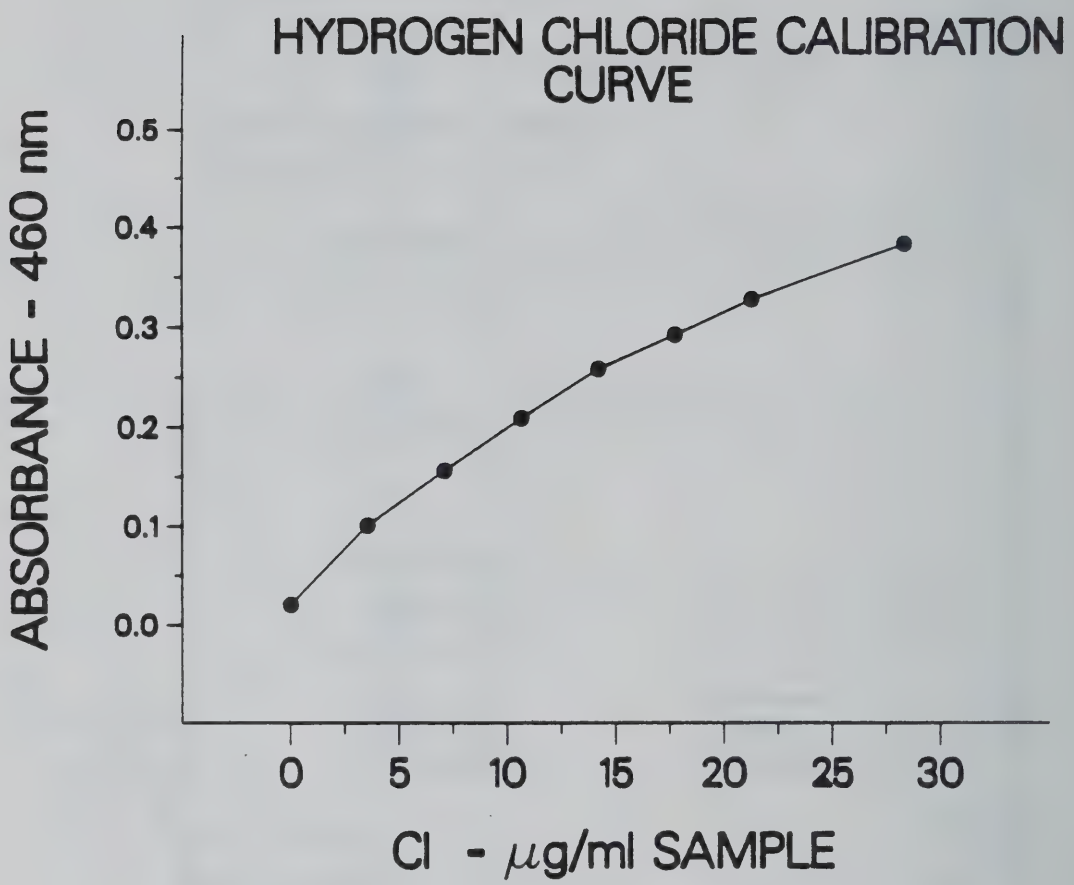


Figure A-3 Calibration curve for HCl assayed by the Osterreiche Stickstoffwerke method (Leithe, 1971).

$$605.3 \times \frac{1 \text{ ppm}}{1.47 \text{ ug } Cl^-} = 411 \text{ ppm HCl}$$

This was the first exposure concentration where running pigs were exposed to HCl. See Methods and Materials.

Appendix B

Exposure Chamber Volume and Concentration-Time Equilibrium Calculations

A. Exposure Chamber Volume Determination

The volume of the ergometer was determined by a dilution technique. A Miran infared analyzer was used as a closed system having a volume to 5,600 ml. An aliquot of carbon monoxide was injected into the system through a septum and was continuously circulated by a pump in series with the system. The injection of gas was measured to be 5250 ppm CO. The pump was then stopped, transport lines disconnected, and then reconnected to the inlet and outlet of the ergometer, now part of the closed system of the pump and Miran. The pump was turned on and the concentration of carbon monoxide was reduced from 5250 ppm to 2700 ppm due to the increased volume of the added ergometer. The volume of the ergometer was calculated.

$$\frac{\text{Miran volume (ml)}}{\frac{\text{Final ppm CO}}{\text{Initial ppm CO}}} = \text{Total volume of ergometer and transport tubes}$$

$$\frac{5{,}600 \text{ ml}}{\frac{2600 \text{ ppm CO}}{5250 \text{ ppm CO}}} = 10{,}895 \text{ ml Total volume}$$

Total Volume (ml) - Miran Volume (ml) - Transport tube volume (ml) = Ergometer volume (ml)

10,895 (ml) - 5,600 (ml) - 395 (ml) = 4,900 ml or 4.9 liters ergometer volume

B. Exposure Chamber Concentration - Time Calculations

In dynamic exposures where a toxicant is continuously being introduced and exhausted, the toxicant in the chamber increases quickly at first from zero, then slowly approaches asymptotically a calculated equilibrium value, the ratio of toxicant flow to total chamber ventilation. Silver (1946) describes this concentration-time curve in the form of an equation:

$$C_t = \frac{f}{F} (1 - \exp - \frac{F}{V} t)$$

Where C_t = Chamber concentration after t minutes of exposure

f = Flow of toxicant

F - Total ventilation of chamber

V = Volume of chamber

This equation assumes that both f and F remain constant and complete mixing occurs in the chamber. The theoretical equilibrium concentration f/F is really never obtained due to the exponential form of the equation. Most commonly the concentration-time characteristics of the chamber are expressed by stating the time required to reach a specific percentage of the equilibrium.

$t_x = k \cdot V/F$

t_x = Time to reach x% of equilibrium concentration

k = A "constant"-the value of which is determined by the value of x

For t_{99}, k = 4.605

t_{95}, k = 2.996

t_{90}, k = 2.303

The guinea pig ergometer was calculated to be 4.9 liters and ventilated by the toxic gas at 2.5 L/min.

$$t_{95} = 2.996 \times \frac{4.9 \text{ L}}{2.5 \text{ L/min}}$$

$$t_{95} = 5.87 \text{ minutes}$$

Therefore 95% equilibration of the ergometer occurs in approximately 6 minutes. The aformentioned theory on concentration-time and equations are described thoroughly by MacFarland, 1983.

Appendix C

Calculation of Nominal Exposure Concentration of CO

Nominal Exposure Concentration Calculation for CO

If a 19,000 ppm CO exposure concentration were desired the required rate of CO delivery (L/min) with the balance air is based on total ventilation and the concentration (ppm) of the standard CO gas. The respective flow rates were calculated as follows:

$$\text{Desired CO ppm} \times \frac{\text{Total ventilation}}{\text{Standard CO gas(ppm)}} = \text{required CO flow (L/min)}$$

Where:

$$\text{Total ventilation} = \text{L/min through Exposure chamber} + \text{L/min exhausted through } 1^{0} \text{ chamber}$$

$$\frac{19{,}000}{} \times \frac{12.50 \text{ L/min}}{1 \times 10^{6} \text{ ppm CO}} = .2375 \text{ L/min CO}$$

Therefore to produce a 19,000 ppm CO exposure approximately .24 L/min of CO and a balance of 12.3 L/min of air are required.

Appendix D

Surgical Procedure for Fitting A Carotid Artery Cannula in the Guinea Pig

A. Pharmaceuticals

A guinea pig was prepared for surgery by an intramuscular injection of both the dissociative anesthetic Ketamine HCl (100 mg/ml) and muscle relaxant Acepromazine Maleate (10 mg/ml) each at a dosage of 0.1 ml/100 grams body weight. This combination of drugs depressed respiration in the guinea pig less than a barbiturate anesthetic would and the animals recovered (walked in their cages) within 45 minutes after surgery.

B. Surgical Procedure

Once the guinea pig was anesthetized, his neck under the chin and back between the shoulder blades were shaved with a fine bladed animal clipper. A 25 mm incision was made through the skin over the area of the carotid artery parallel to either side of the trachea. A 6 mm incision was also made between the shoulder blades. A cannula (250 mm long PE 10 intramedic polyethylene tubing) with attached 30 G needle and saline filled syringe (1 ml) first threaded a plastic cap (previously used to cover the tip of a plastic syringe during packaging) and then was passed under the skin from the cut on the guinea pig's back to the incision near the carotid. The animal was then placed on his back, each limb secured by rubber bands at posts at each corner of a 10" x 7" surgery board. As surgery was performed, the head of the guinea pig was towards the surgeon. The carotid artery was isolated with forceps, dialated with a bath of 10% Xylocaine and looped with 2 pieces of silk thread each with a single throw of a knot. A plastic straw was placed under the artery to lift and further isolate it. One of the threads (that furthest from the head) was lifted, to crimp the artery and stop

the flow of blood. A very small hole was made in one wall of the artery. The slightly beveled free end of the catheter was held by curved forceps and inserted into this hole and carefully advanced 20 mm into the artery towards the heart. The attached syringe was aspirated to determine a free blood flow, then the catheter was flushed. The cannula was then secured in the artery by a drop of Crazy Glue^{R}. The way it was positioned could be compared to a straw sealed in the side of a garden hose. Blood that was pumped from the heart could either be aspirated into the cannulated or flow passed the catheter to feed the brain. The cannula was further held in place by loosely tying the 2 silk threads, and looped to provide slack in the line. The incision over the carotid was closed by several sutures. The plastic cap was glued in the 6 mm cut between the guinea pigs shoulders. The cannula was crimped temporarily so that the needle and syringe could be removed without blood coming through the cannula. Then the end was plugged with hematocrit sealing clay. The excess length of cannula exiting from the guinea pig's back was then uncrimped, coiled and protected with tape so it could not be easily chewed.

C. Blood Sampling During Exposure to CO

When the guinea pig was prepared the following day for an exposure and coincident blood sampling, the cannula was uncoiled, passed through a septum in the chamber, its clay seal snipped, and a saline-filled syringe and needle were attached. A small volume of saline (.2 ml) was injected into the catheter to clear the line then aspirated again to access blood flow, and then reflushed. Heparin (10,000 units/ml) coated tuberculin plastic syringes were used to sample blood from the guinea pigs at S-incapacitation during carbon monoxide exposures. At the time

of actual sampling, blood was aspirated with a saline filled syringe to see significant contamination of the saline, then a heparinized syringe was attached and 1 ml of blood was withdrawn. The time (min) required to take the blood was recorded. The volume was replaced by 1 ml of saline. The sample was capped and rolled between the hands to insure adequate mixing of the blood and anticoagulant.

Appendix E

Analytical Method for Carboxyhemoglobin Measurement In the Guinea Pig

A. Method Source

The procedure to be described is a modification of the method for analysis of %COHb in human blood at the Allegheny County Department of Laboratories. Technical advice was provided by Mr. Frank Esposito who is not only employed by this laboratory but is also a fellow doctoral student in Toxicology.

B. Basic Principle

Whole blood is hemolyzed with ammonium hydroxide. Hydrosulfite is added to convert O_2Hb and MetHb to Hb but has no effect on COHb. The absorbance of COHb pigment is measured spectrophotometrically at 539, 554, and 579 nm. The calculated ratios of $\frac{Ab_{539}}{Ab_{554}}$ and $\frac{Ab_{539}}{Ab_{579}}$ and corresponding % COHb is determined from a standard curve.

C. Reagents Required for Analysis

1. 0.1 M NH_4OH
2. Saturated sodium borate solution
3. Sodium hydrosulfite

D. Blood Analysis

1. 0.2 ml of whole blood was placed in a small test tube
2. 0.5 ml .1 M NH_4OH was added to tube and mixed well
3. Wait for 5 min.
4. The tips of 4 hematocrit tubes were filled with sodium hyposulfite, added to the test tube sample of blood, and mixed well.

5. The tubes were covered and centrifuged at a medium speed for 5 min.
6. A Perkin-Elmer (Lamda 4B) uv/vis spectrophotometer was used to measure absorbence against a blank at 539, 554 and 579 nm. The instrument was operated with the following settings:

min/max	0.0 - 0.1
wavelength	400-600 nm
scan	120
slit	1
response	3

E. Determination of Absorbences Used Specifically for Guinea Pig Blood

Spectrophotometric methods for the measurement of COHb in human blood have used the wavelengths of approximately 540, 555 and 579 nm (Tietz and Fiereck, 1973). When the 0% COHb guinea pig blood was scanned from 400 to 600 nm the absorbence peaked at 554 nm, two peak absorbences occurred at 569 and 539 nm while scanning 100% COHb prepared blood. The absorbence at 579 nm was simply adopted aritrarily as a reference value.

F. Calibration Curves for COHb

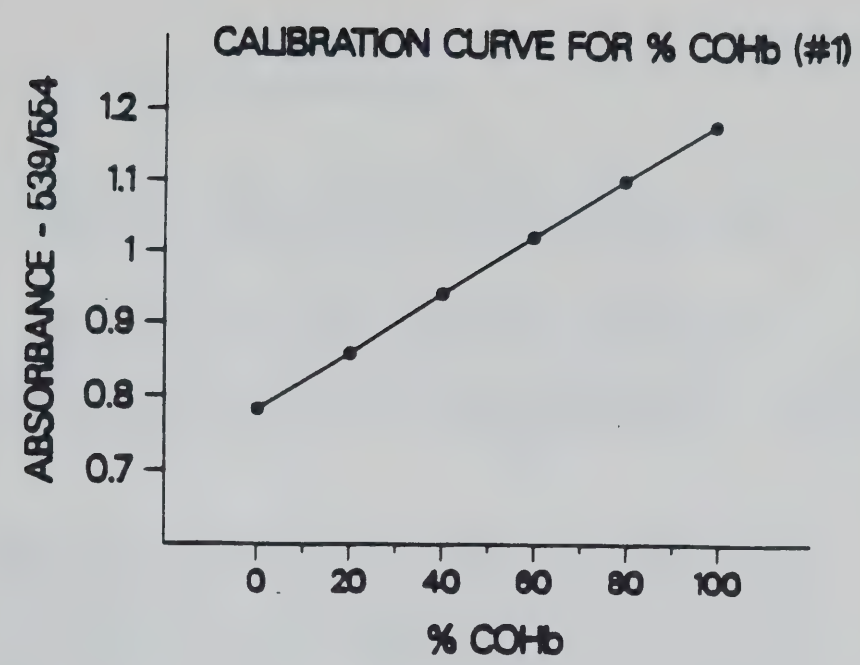
Five guinea pigs were anesthesized and blood was taken from their inferior vena cava to collect a total of 30 ml. This volume was mixed well, 15 ml remained untreated (0% COHb) and 15 ml was placed in a 60 ml plastic syringe and capped. Carbon monoxide gas from a 10% tank was introduced into the syringe, the syringe capped, twirled for 5 minutes

then the CO was released. This procedure was repeated approximately 5 times, the purpose of which was to fully saturate this 15 ml aliquot of blood with CO (100% COHb). Different proportions of each of these bloods were mixed to prepare six different standard tubes of blood with theoretically known values of COHb. These were prepared as shown in Table E-1. Each of these standards were analyzed by the procedure previously described. Two calibration curves (Figure E-1) were generated, the nominal %COHb in each standard was plotted against the absorbance values at $\frac{539}{554}$ and $\frac{539}{579}$. When a blood sample was analyzed %COHb was read from each curve, the two values were then averaged.

TABLE E-1

Carboxyhemoglobin standards for the analysis of guinea pig blood

Standard No.	Nominal % COHb	100% COHb (ml) Prepared blood (ml)	0% COHb (ml) Blood (ml)
1	100	2.0	0.0
2	80	1.6	0.4
3	60	1.2	0.8
4	40	0.8	1.2
5	20	0.4	1.6
6	0	0.0	2.0



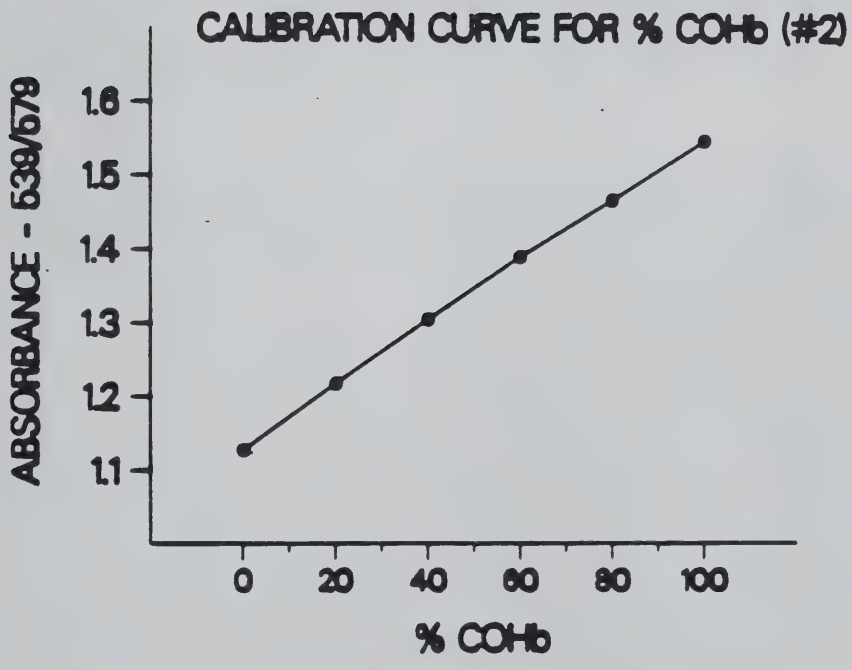


Figure E-1 Calibration curves for %COHb for the guinea pig. Standard blood samples with known %COHb are plotted against the ratio of absorbance values at 539/554 and 539/570.

APPENDIX F

"Mouse Track Model"

FUNDAMENTAL AND APPLIED TOXICOLOGY 8, 335–345 (1987)

Performance Evaluation under Intoxicating Atmospheres

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Performance Evaluation under Intoxicating Atmospheres. MALEK, D. E., STOCK, M. F., AND ALARIE, Y. (1987). *Fundam. Appl. Pharmacol.* 8, 335–345. A new behavioral model has been developed and used to assess the performance of mice during exposure to carbon monoxide, hydrogen chloride, or subambient levels of oxygen. The apparatus is a ventilated 150-ft series of glass tubes forming an S-shaped exposure system. Performance evaluation was obtained for two sublethal responses: (1) distance traveled/time and (2) incapacitation. Performance of normal mice (Type I) or mice previously fitted with a tracheal cannula (Type II) was very reproducible and similar. Concentration–response relationships were obtained showing the deterioration of performance with exposures to CO from 2500 ppm, HCl from 1095 ppm, and below 8.8% ambient O_2 level. This model is likely to be sensitive to other asphyxiants and irritants. It includes both distance traveled and time of performance prior to incapacitation. Both are critical parameters to be included in escape hazard analysis in fire situations and possibly in other accidents involving chemical spills. © 1987 Society of Toxicology.

During confined space fires, a number of factors such as thermal stress, a reduction in visibility, the development of fear, and the toxic effects of gases and aerosols can interfere with escape and survival of humans.

The need to characterize the toxicity of thermal decomposition products has fostered the development of animal tests to assess the relative toxicity of smoke from different materials during thermal degradation. Current combustion toxicity tests, notably those at the National Bureau of Standards (Levin *et al.*, 1982) and the University of Pittsburgh (Alarie and Anderson, 1981), are largely based on a lethality endpoint; however, sublethal effects, and time to reach a given effect, have also been studied (Hartung *et al.*, 1977; Hilado and Cumming, 1977; Packman *et al.*, 1978; Kishitani and Nakamura, 1979; Kaplan *et al.*, 1985).

Sublethal effects for the most part have been investigated by various animal behavioral methodologies. Upon exposure to toxicants an animal's capability to perform a previously acquired task is examined. These tasks include the hind foot flexion reflex (Packham *et al.*, 1978), balancing on a rotating rod to avoid presentation of electric shock (Hartung *et al.*, 1977), collapsing in a rotating cage (Kishitani and Nakamura, 1979), and most recently, escaping by an appropriate lever press (Kaplan *et al.*, 1985). The term "incapacitation" has been commonly adopted to describe the inability, as defined by the criterion of a test method, to perform a designated task or perform it in a specified period of time. The intended utility of sublethal endpoints is to assess the escape potential of animals exposed to fire gases and use this data to predict human response.

The two major categories of toxicants involved in impairing escape are the asphyxiants such as carbon monoxide, hydrogen cyanide, and reduced oxygen levels, and the irri-

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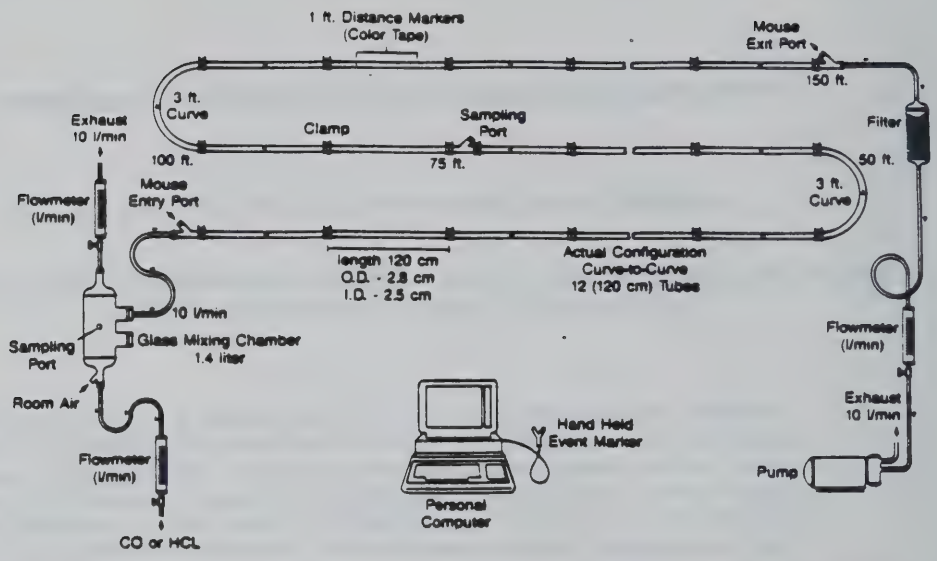


FIG. 1. 150-ft mouse track exposure system. Made from a series of 4-ft sections of straight and 3-ft sections of curved Pyrex glass tubes. Sections were joined together by clamps at their ball joint connections. Y-tube added for entry, sampling, and exit port as shown. The pollutants CO or HCl were metered for mixing with room air as shown. Nitrogen was also similarly metered to reduce O_2 concentration.

tants, such as hydrogen chloride and acrolein (Alarie and Anderson, 1979). The behavioral models used in combustion toxicity studies, for the most part, are sensitive to the asphyxiant carbon monoxide but show a lack of sensitivity for the irritants. Animals exposed to irritants are often very close to death at incapacitation, if incapacitation even occurs by the definition of a particular model (Levin *et al.*, 1982; Kaplan *et al.*, 1985). A model dependent on respiratory pattern analysis (Matijak-Schaper and Alarie, 1982; Alarie and Anderson, 1979; ASTM, 1984) was found to be sensitive to both asphyxiants and irritants, but it is a nonbehavioral model. To assess escape potential from a toxic environment more confidently, a model should test overall performance. A behavioral performance model has been introduced by Wood (1979) and Tepper *et al.* (1985) which appears to be sensitive to irritants and with modifications could also be sensitive to asphyxiants.

The objective of this paper is to introduce a new behavioral animal model, featured with two escape predictive responses: (1) distance traveled/time, and (2) incapacitation.

METHODS

Animals. All animals utilized in this study were outbred specific pathogen-free male Swiss Webster mice ranging in body weight from 15.5 to 17.5 g and were purchased from Hilltop Labs, Inc., Scottdale, Pennsylvania. They were fed Purina Mouse Chow and tap water *ad libitum*. The mouse was chosen because the performance of this species is likely to be related to changes in respiratory pattern and rate, among other variables, as previously reported during exposure to fire gases (Alarie and Anderson, 1979; Matijak-Schaper and Alarie, 1982). In addition to their ease of husbandry and low cost, mice are notably curious and will run without provocation as described below.

The performance of 8 mice was evaluated at each gas exposure concentration and that of 20 mice of each of the two major categories of mice was evaluated during exposure to air (control). The categories of mice were of two types:

Type I: Each of these mice was weighed, placed into the exposure system, and exposed to a single concentra-

tion of toxic gas without any form of pretreatment. These animals were also referred to as "noncannulated" mice.

Type II: Prior to hydrogen chloride exposures these mice were anesthetized and fixed with tracheal cannulas according to the protocol outlined by Matijak-Schaper and Alarie (1982). This procedure eliminated absorption of hydrogen chloride by the nasal mucosa and facilitated the introduction of gas directly into the lower respiratory tract. These animals were referred to as "cannulated mice." Within an hour postanesthesia to introduce the tracheal cannula, the animals were active and moved freely about their cage. This observation although somewhat subjective was the criterion we used to begin the training of the mouse for subsequent exposure.

Mouse-tract exposure apparatus. A simple model was designed to estimate behavioral performance and it consisted of a 150-ft series of 4-ft sections of straight and 3-ft sections of curved Pyrex glass tubes and Y-shaped tubes joined together by clamps at their ball joint connections (Fig. 1). A 150-ft track was chosen arbitrarily as during the development of this model mice were observed to run at a fairly constant rate to distances greater than 300-ft.

The inside diameter of the tubes was 2.5 cm, large enough to accommodate and allow an unrestricted forward passage of a mouse and small enough to prevent the mouse from turning around and moving in the opposite direction. Colored strips of tape on the glass demarcated each foot of the system. The track was an elongated S-shaped curve formed by the two bends of tubing at the 50- and 100-ft marks, respectively. There were three Y tubes in the track and were located at the zero, 76-, and 150-ft marks. The one at foot zero was used for the introduction of the animal into the system, the 76-ft Y-tube served as the port for sampling exposure gas for chemical analysis, and that at 150 ft was used to remove animals following exposure as well as for sampling exposure gases for analysis.

A 1.4-liter glass mixing chamber was located at the front of the track and was used to produce a desired exposure concentration of gas by combining appropriate proportions of the gas to be tested and air. When the track system was in operation a total flow of 20 liters/min was passed through the mixing chamber. Each minute 10 liters of gas was immediately exhausted to a ventilation duct, and another 10 liters of gas was pulled through the track (at a speed of 72 ft/min) by a rotary vane pump located beyond the 150-ft mark. These flows were regulated by valves and monitored by flow meters.

The forward progression of the test mouse in the 150-ft system was monitored by a computer which acted primarily as a time base. Throughout the exposures each distance of 1 ft made by the animal was registered relative to time by a depression of a hand-held event marker. When the exposure was terminated the cumulative feet traveled by the mouse were calculated for every 10-sec exposure time.

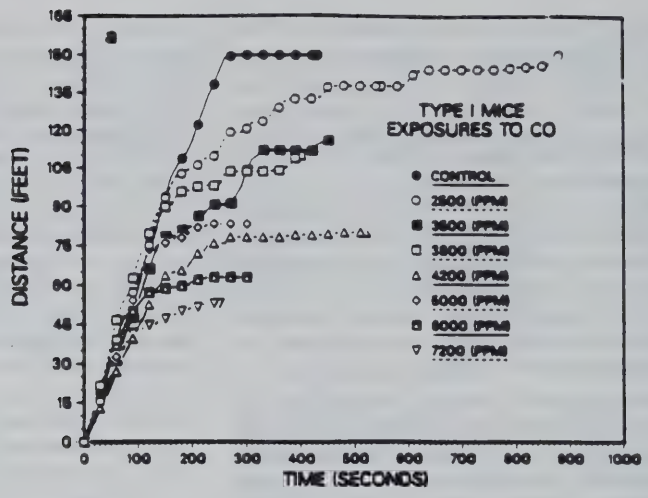
Animal training and exposure. Each mouse, whether it was a "test" animal exposed to a toxic gas or a "control" animal exposed to air only, was introduced into the tract for three separate runs. When first introduced it would spontaneously move forward into the tube and travel toward the end. The distance traveled by the mouse relative to time was measured during each trial through the track.

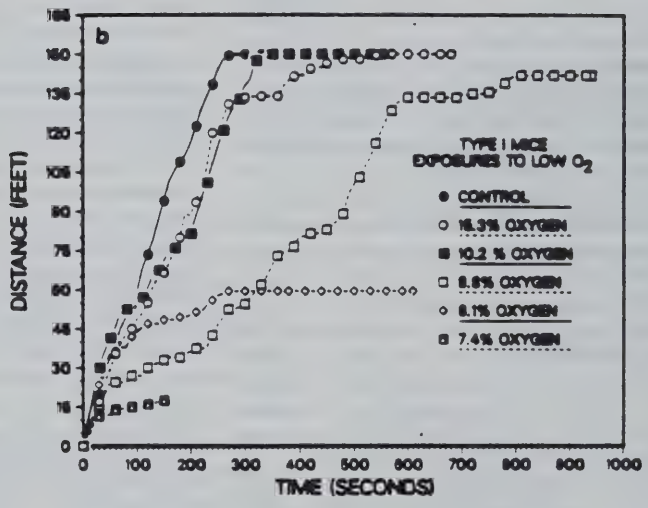
The first trials of the animals were somewhat erratic, with the animal moving forward, stopping, moving backward, and moving forward again. After a rest period of 5 min the animal was reintroduced into the tube, and movement forward was much less erratic. Again the animal was permitted to rest and was then reintroduced into the tube for a third time. On this third trial forward movement was very good and travel speed was measured once again. The results of this third trial were used as control performance. Control data were collected for both Type I and Type II mice.

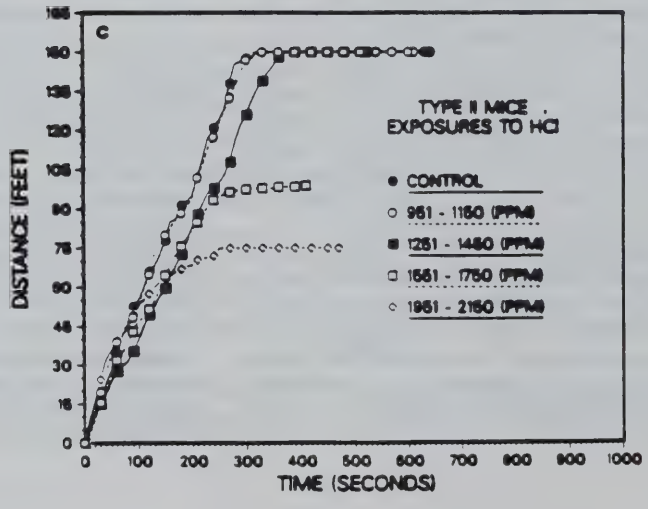
To investigate the effect of toxic gases on travel performance the animal, as in the control groups, was permitted to travel through the tube with only the flow of air for two trials. If the time to reach 150 ft on their second trial in air was within the mean and one standard deviation of the control group, the animal was used. Twenty percent of the animals were rejected. If the animal met this criterion, the animal was allowed to rest and the gas of interest was pulled through the track for approximately 10 min. When the gas was of the desired concentration the animal was introduced into the track for a third time and exposed. Its speed was monitored. The exposure time was defined by one of two events: when the mouse either reached 150 ft or became incapacitated. Incapacitation by this model occurred when a mouse slowed its stride or stopped and failed to progress more than 3 ft in 3 min time.

Analysis of exposure concentration. The gases utilized in this study included the asphyxiants nitrogen and carbon monoxide and the irritant hydrogen chloride. Elevated nitrogen–low oxygen environments were produced by using compressed nitrogen (Matheson). The exposure concentrations were monitored continuously by a Beckman oxygen analyzer and ranged from 15.3 to 7.4% O_2. Carbon monoxide was measured continuously with a Miran infrared analyzer. The carbon monoxide exposure concentrations in this study ranged from 2500 to 7200 ppm. Hydrogen chloride exposures ranged from 950 to 2150 ppm. This gas was sampled at 0.3 liters/min through two midget impingers containing 0.1 N NaOH. Normally two 2-min samples were taken at 2 and 5 min into the exposure as the mouse moved through the track. The samples were then analyzed by the Osterridischen–Stickstofferwerke method as described in Leithe (1971).

In the development of the exposure system, analytical measurements were taken at various points along the track and were found to be consistent; consequently, the 76-ft port was adopted for routine gas sampling.


a
TYPE I MICE
EXPOSURES TO CO
CONTROL
2500 (PPM)
3600 (PPM)
3900 (PPM)
4200 (PPM)
6000 (PPM)
6000 (PPM)
7200 (PPM)
DISTANCE (FEET)
TIME (SECONDS)


b
TYPE I MICE
EXPOSURES TO LOW O_2
CONTROL
16.3% OXYGEN
10.2 % OXYGEN
8.8% OXYGEN
8.1% OXYGEN
7.4% OXYGEN
DISTANCE (FEET)
TIME (SECONDS)


c
TYPE II MICE
EXPOSURES TO HCl
CONTROL
951 - 1150 (PPM)
1251 - 1450 (PPM)
1551 - 1750 (PPM)
1951 - 2150 (PPM)
DISTANCE (FEET)
TIME (SECONDS)

Evaluation of performance. The following measurements were taken when applicable during each mouse exposure either to air only or to a toxic gas.

(a) Distance traveled (feet)/time (seconds)

(b) Occurrence of incapacitation

(c) Total distance traveled (feet)

(i) 150 ft, or

(ii) distance before incapacitation

(d) Time to reach distance traveled (seconds)

(i) time to 150 feet, or

(ii) time to incapacitation

After eight animals were successfully exposed to the same concentration of gas or control groups (Type I and Type II) of 20 mice each were exposed to air, the following calculations and relationships were made where appropriate.

(a) Median distance traveled (feet)/time (seconds)

(b) Mean total distance ± SD traveled (feet)

(c) Mean time ± SD to 150 ft (minutes)

(d) Percentage mice that traveled a distance less than 100 ft

Finally where appropriate these responses were related to exposure concentration and analyzed using linear least-squares regression analysis described by Armitage (1971).

RESULTS

Control Air Exposures

The means ± SD times to travel the 150-ft track for Type I and Type II control animals were 5.0 ± 1.3 and 5.8 ± 2.3 min, respectively. No statistical difference was found between the mean times of these mice where groups of 20 animals each were compared.

Traveled Distance/Time

Figure 2 represents the distance traveled/time data that can be generated in the model described above. Each point represents the median distance (feet) traveled in time (seconds) by 20 Type I or Type II control mice or groups of 8 mice exposed to various concentration of carbon monoxide, low O_2, or HCl. Each mouse of a particular exposure group was exposed, one at a time, as it moved through the track system and the median distance traveled was calculated. The median distance/time curves in Fig. 2 are based on eight animals until a mouse terminates its exposure either by becoming incapacitated or by traveling the entire 150-ft track. Therefore, as the curves were plotted the number of animals used to calculate the median distance traveled/time decreases. The last point on each curve represents the time when the last animal of a defined exposure group ended its toxic exposure. Although the mice associated with these times were no longer being exposed after being incapacitated, their individual total distance traveled value was included in all subsequent calculations of median distance traveled/time.

Total Distance Traveled

From the data presented in Fig. 2, the total distance traveled at each exposure concentration can be obtained and plotted as shown in Fig. 3. The mean distance traveled decreased as the concentrations changed.

Mean Minutes to Incapacitation

Of those animals in specific exposure groups that were incapacitated, the mean time to incapacitation vs the exposure concentration was calculated and the results are presented in Fig. 4. Similar to distance trav-

FIG. 2. Distance traveled/time relationships in control animals (Type I and Type II) and during exposure to (a) CO, (b) low O_2, or (c) HCl. Each point represents the median distance traveled under each condition, using 20 animals for Type I and Type II controls and 8 animals at each concentration for CO, low O_2, and HCl.

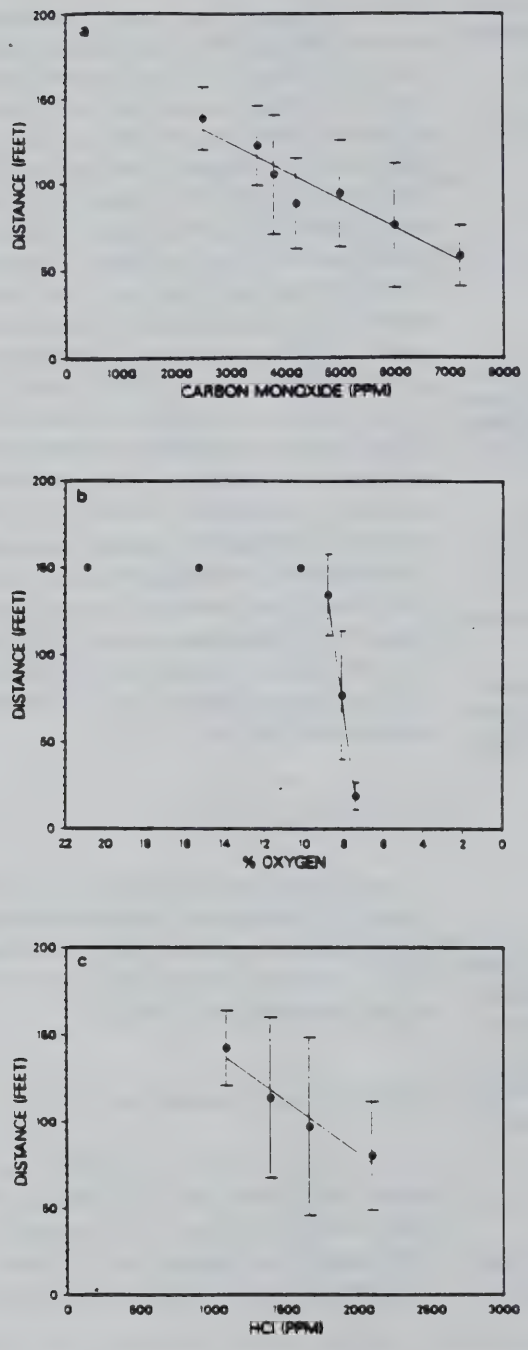


FIG. 3. Mean distance traveled ± SD prior to incapacitation by groups of mice ($n = 8$) during exposure to (a) CO, (b) low O_2, and (c) HCl. Curves fitted by linear least-squares analysis, slope significantly different from zero for all cases, and r^2 higher than 0.91 for all cases.

eled the mean time to incapacitation changed with exposure concentration. However, with HCl no concentration–response relationship was found. At all concentrations of HCl tested, all mice were incapacitated at about the same time (4.4 min). Also, three animals at the highest concentration died within 1 min of incapacitation.

Percentage Failing to Travel 100 ft

A concentration–response relationship may be observed as shown in Fig. 5 where the percentage of animals of an exposure group that traveled the track less than 100 ft is plotted against exposure concentration.

DISCUSSION

Compared to other behavioral animal models designed to evaluate performance capability during exposure to components of fire smoke, the mouse track model described appears to be unique with respect to the nature and potential application of its sublethal responses.

Concentration–response relationships have been developed in various models by use of an incapacitation endpoint. In such modes animals either perform a task at a defined level or they are considered incapacitated. Attempts have been made to observe and quantitate behavioral changes that occur prior to incapacitation that demonstrate levels of compromised performance. These include scored parameters in rats such as "lag and turn" in the activity/tumble cage model (Boettner *et al.*, 1978), avoidance failures and time to avoidance deficits (TAD) in the rat pole-climb conditioned avoidance/escape test (Dilley *et al.*, 1979), and various response parameters such as time to first response or first correct response, number of correct or incorrect responses, and "avoidance" in the baboon model of Kaplan *et al.* (1985). Avoidance failures for example are thought to re-

flect disorientation, sensory deficits, and/or motor impairment. None of these however provide a minute by minute profile of animal performance upon exposure to a toxicant as does the mouse track model. The model proposed by Tepper *et al.* (1985) is amenable to this profile and revolution/minute could be converted to distance traveled.

The distance traveled/time parameter is a sublethal response and most relevant to an escape process. Unlike other models such as the foot flexion model (Packham *et al.*, 1978), the rotored model (Hartung *et al.*, 1977), and the greased pole model (Dilley *et al.*, 1979) where failure to perform a conditioned task (incapacitation) can only be indirectly related to the inability to escape, the distance traveled/time sublethal response is a response of interest when examining performance.

Concentration-dependent–distance traveled/time curves have been developed for the evaluation of performance under intoxicating levels of carbon monoxide, reduced oxygen, and hydrogen chloride. These curves demonstrate how quickly these asphyxiating and irritating conditions impair performance. For each toxicant, the initial speed of the exposed animals is nearly the same as the speed of the controls; however, it soon declined as the exposure proceeded. A graded response was observed until a concentration-dependent–distance traveled/time plateau was reached. We can use these curves to determine the maximum permissible exposure concentration to move a required distance in a designated period of time. In the case of CO if moving 100 ft in approximately 3.5 min (210 sec) were required, the maximum permissible exposure concentration would be 3800 ppm. Given the same distance and time for reduced oxygen or HCl, the maximum permissible concentrations are 15.3% and the range 1251–1450 ppm, respectively.

Aside from these performance curves additional approaches have been developed with the data generated in this model to evaluate the escape-impairing capacity of toxicants

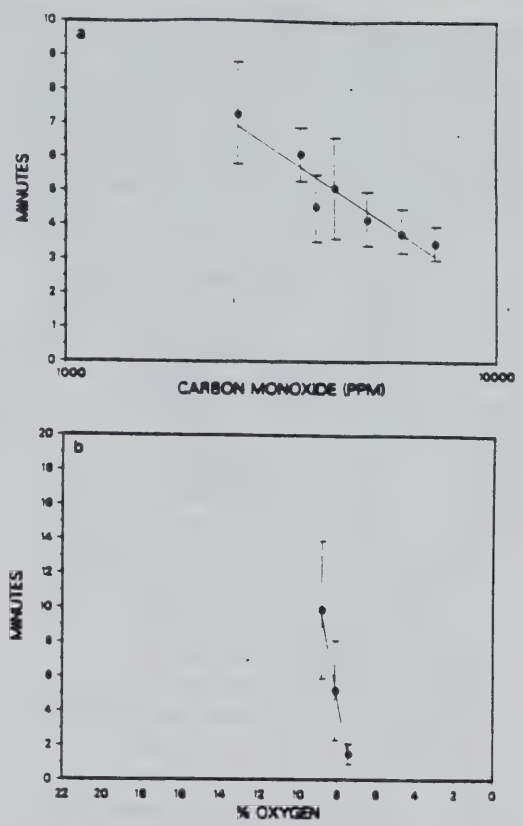


FIG. 4. Mean minutes ± SD to incapacitation of mice during (a) CO or (b) low O_2. The mean is given for those incapacitated at each concentration and varied from $n = 3$ to $n = 8$ from low to high CO and $n = 4$ to $n = 8$ from high to low O_2.

effectively. These include (1) the mean distance traveled, (2) the percentage that failed to travel a specified distance, and (3) the mean time to incapacitation.

The utility of the concentration/mean distance traveled relationship is as follows. If a mean distance traveled of 100 ft were selected from each of these curves, a corresponding concentration of toxicant responsible for this incapacitation can be determined. These concentrations are approximately 4500 ppm, 9.7%, and 1800 ppm for CO, reduced O_2, and HCl, respectively.

Another approach to compare the performance-impairing capacity of toxicants is to

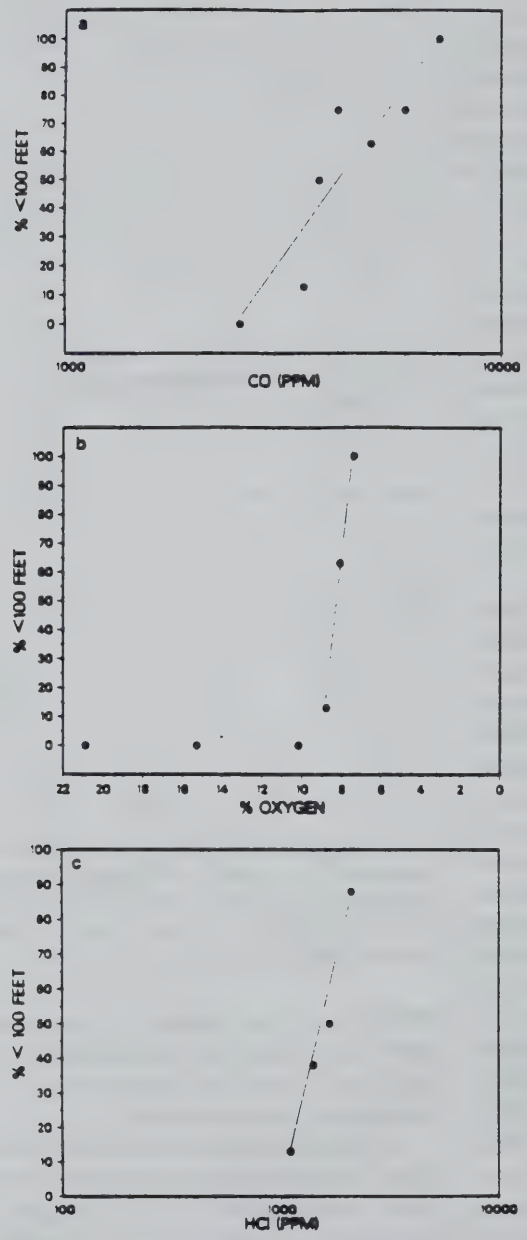


FIG. 5. Concentration–response relationship showing the percentage of animals traveling less than 100 ft during exposure to (a) CO, (b) low O_2, and (c) HCl.

again choose a particular distance and determine the percentage of those of an exposure group that was incapacitated before this distance, as we did by selecting 100 ft. From these curves the concentrations, for example, that incapacitated 50% of an exposure group before 100 ft (or any other distance) could be compared.

Time is a critical factor in the escape process. We have quantitized not only distance traveled in time but also, as a third approach to performance evaluation, minutes to incapacitation. To review the definition of incapacitation in this model, it is the event when an exposed animal stops or progresses less than 3 ft in 3 min time. It is a definitive, nonsubjective endpoint directly related to escape. For the asphyxiants CO and reduced $\%O_2$, incapacitation not only occurred but the time to incapacitation was concentration dependent. These findings are consistent with the data obtained in other models (Kishitani and Nakamura, 1979; Matijak-Schaper and Alarie, 1982; Kaplan *et al.*, 1985). For CO the CT (exposure concentration, C, times time to incapacitation, T) calculated from our data is fairly constant (17,100–25,200 ppm-min) but somewhat lower than that reported in other test systems including human studies where the average CT is about 35,000 ppm-min (Steward *et al.*, 1973; Hartung *et al.*, 1977; Kaplan *et al.*, 1985). This is to be expected since CO uptake in mice is faster than in larger animals and humans (Haldane, 1895).

In the mouse track model no incapacitation was observed in animals exposed to oxygen levels above 10.2%. This is not surprising due to the nature of the oxygen dissociation curve. Minimal alteration in oxyhemoglobin saturation occurs since arterial PO_2 reduction resulting from reduced oxygen exposures rests within the plateau region of the sigmoid curve (West, 1979). Below 8.0% the oxygen dissociation curve is steep. Thus, incapacitation occurred in nearly all animals exposed to oxygen below 8.8%. Incapacitation was very rapid (2 min) at 7.4% O_2. Death occurred almost immediately after incapacitation (30 sec) at con-

centrations below 7.4%. This effect was more rapid than evidence of asphyxiation in the Matijak-Schaper and Alarie model (1982) or collapse in the Kishatani and Nakamura model (1979), where mice were also used. Thus, the mouse track system is a more sensitive model for subambient O_2 probably because of the exercise involved as compared to sedentary mice in the two other models.

In HCl-exposed mice, incapacitation occurred in a concentration-dependent fashion and at the highest exposure range three animals died within a minute of incapacitation. This was not observed at any other exposure concentration but the monitoring of postincapacitation lethality during continued exposure was not pursued in this study. The close proximity of the occurrence of incapacitation to the occurrence of death upon exposure to irritants with respect to concentration and time is a common disadvantage among current escape models (Levin *et al.*, 1982).

In order to investigate the effect of HCl, we adopted the use of tracheal cannulated mice. The protective high-scrubbing efficiency of the mouse nose for this water soluble and reactive gas has been demonstrated in previous studies (Anderson and Alarie, 1980). The time required for surgery is the only limitation of the cannulation procedure. Although there is a concern about the adverse effects of anesthesia and surgery, these animals (Type II) were not significantly handicapped compared to normal (Type I) mice. An important advantage of the cannulated mice is that they stimulate human breathing conditions during a fire. Toxicants which would be effectively scrubbed by the nasal mucosa in mice are deposited more directly into the lower respiratory tract. A most significant finding in our study of HCl with cannulated mice is that they are rapidly incapacitated and near death upon exposure to 1000–2000 ppm HCl, a range reported to be "dangerous for even short exposure" for humans (Henderson and Haggard, 1927). Flury and Zernik (1931) summarized the work of several investigators on the effect of HCl on a variety of laboratory animals and humans. They are in agreement with the statement of Henderson and Haggard and stated that accustomed human subjects can tolerate 670–1250 ppm for several minutes; however, swelling and closure of the vocal cords prohibit longer tolerance because of suffocation. In the review of the literature on HCl for the National Academy of Sciences (1976) the committee also proposed that 1000–1300 ppm would be dangerous for humans for a period of exposure of 30–60 min, while 1300–2000 ppm would be lethal within a few minutes. It is likely that our cannulated mice were incapacitated by bronchospasm induced by irritating HCl. Since the cannula was placed below the larynx no laryngeal spasm could have been induced. Recently Kaplan *et al.* (1985) developed a baboon escape model at concentrations of HCl from 196 to 17,290 ppm. By definition of their model, regardless of the concentration and severity of clinical observations which included agitation, frothing at the mouth, gagging, coughing, and dyspnea, all animals escaped and, therefore, were not incapacitated until the very high concentrations. Their escape, however, was a simple task. These investigators opposed the recommendations of Henderson and Haggard (1927) and feel that "dangerous" does not indicate incapacitation. By the design of their model, regardless of their failure to be incapacitated it is clear that these baboons were seriously injured and compromised. More recent studies by Kaplan *et al.* (1986) using pulmonary function tests and lower concentrations of HCl may provide better answers to the incapacitating nature of this gas in baboons.

In order to predict escape capability of humans from the performance data of mice generated in our model, it is necessary to understand the relative energy costs required of

these species to travel similar distances. A distance traveled of 1 ft is a significant expenditure of energy (in terms of oxygen consumption) and is an accomplishment for a mouse and is not the same for a human. Taylor *et al.* (1970) has demonstrated a linear relationship between oxygen consumption (ml O_2 kg^{-1} hr^{-1}) and running speed (km hr^{-}) for several species of animals. Since the cost of running, expressed in oxygen per kilogram per kilometer (slope) for each animal is independent of the speed at which it runs, speed as a variable can be eliminated in the calculation of cost. Therefore cost of running can be directly related to body mass. The work of Taylor (1970) and more recently the work of Schmidt-Nielsen (1984) have shown an inverse linear relationship between cost and body size among various birds, reptiles, and mammals including humans. Cost can be calculated from the equation

$$\text{Cost} = aM_b^b,$$

where M_b is body mass in kilograms, b is the exponent (slope), and a is the intercept at unity. If one were to use the mean exponent of −0.32 reported by Schmidt-Nielsen (1984) and 8.46 as the intercept arbitrarily taken from Taylor (1970), the cost of a 0.017-kg mouse is greater than 10× that of a 70-kg human traveling the same distance. In effect the larger the animal the more efficient he is. Furthermore, a mouse is a very sensitive animal model for inhalation toxicity studies due to its high minute ventilation. Thus, conservative estimates of toxic limitations for human exposures could be arrived at using the approaches described. The most useful addition of the model is the capability of assessing a deterioration in performance over a very short period of time, instead of relying on an all or nothing incapacitation response, and to be able to measure it within a relative short time period relevant to fire escape situations.

ACKNOWLEDGMENTS

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11. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)

Previously the toxicity of fire smoke has been examined primarily in sedentary animals and lethality was noted. The evaluation of escape potential from a toxic environment, however, requires the measurement of sublethal responses in active animals that are escape predictive. To address this need the mouse track model and the guinea pig ergometer model have been developed.

The mouse track model was a ventilated "S" shaped exposure system. Performance was evaluated by two sublethal responses, distance traveled/time and incapacitation. At seven concentrations of CO (2500-7200 ppm) and four concentrations of HCl (1095-2095 ppm) mice were evaluated and compared to control mice. Performance was impaired within two min for both CO and HCl at all concentrations tested. A novel feature of the mouse track model was its ability to detect an early deterioration in performance before incapacitation and death. The guinea pig ergometer model was designed where a 4.9L exposure chamber enclosed a moter driven rubberized wheel. Extrapolation of exercising guinea pig data to human was similar to theoretical models that predict human response to CO. Humans were estimated to progress five times the distance of the guinea pig at a similar level of toxicity for CO.

12. KEY WORDS (Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)

animals; carbon monoxide; human behavior; hydrogen chloride; toxic gases; toxicity test methods

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